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WILT OF GIPSY-MOTH CATERPILLARS[1]

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INTRODUCTION

The present investigation of the wilt of gipsy-moth caterpillars (*Porthetria dispar* L.) was undertaken in the hope of obtaining results of economic importance. Two questions that have caused considerable speculation are, When did wilt first appear in the United States? and How did it get here? The gipsy moth was brought from France to Medford, Mass., in 1869, but it did not become a very serious pest before 1889, when active suppression work was begun by the State of Massachusetts. However, there is no account of the appearance of wilt prior to 1900, although old State records and documents have been gone over very thoroughly. The writer has talked to a number of people, but no one seems to have seen wilt before 1900. Among these was Mr. C. W. Minott, who has had much experience with the gipsy moth since 1894. In response to an inquiry of the writer in regard to the history of wilt, Prof. Charles H. Fernald, of the Massachusetts Agricultural College and formerly entomologist of the Massachusetts State Board of Agriculture, wrote as follows:

> From the first noticeable outbreak of the gipsy moths in Medford in 1889 up to 1900, when the legislature closed the work, I was in close touch with it and spent all the time I could spare from my college work here. During that time I neither saw nor heard of the "wilt" disease nor of anything in any way resembling it. I went down there every week and was around with the men in the field in every part of the infested region, and if either they or the field director had noticed anything of the kind they would surely have told me, for they all knew that we were hunting for and breeding all the parasites we could find.

[1] The writer desires to express his thanks to those who rendered him valuable assistance in his work: Prof. William M. Wheeler, for his encouragement and advice; Prof. Charles T. Brues, for his helpful criticisms; Mr. A. F. Burgess, Director of the Gipsy-Moth Laboratory, Melrose Highlands, Mass.; Dr. J. W. Chapman, who assisted the writer in investigating the etiology of the wilt disease; Miss Teresa Sheerin; and Mr. J. J. Culver.

How the wilt of gipsy-moth caterpillars was brought to this country will probably always remain a puzzle, but many possibilities suggest themselves. Wilt and *Wipfelkrankheit*, a disease of the European nun moth caterpillars, may be identical, and the disease may have been introduced on trees and shrubbery or other material. This is not at all improbable, for when caterpillars die and disintegrate on trees, the virus may dry on them, making it easy for the disease to gain entrance into this country on shipments of plants. This seems very likely in the light of recent investigations by Escherich and Miyajima (4) and Prowazek (12) on the long resistance to drying of the virus of Wipfelkrankheit and Gelbsucht.

Then, again, wilt may have been introduced from its original source with the parasites in 1905, when the State of Massachusetts, in cooperation with the Federal Bureau of Entomology, imported large numbers of parasites and natural enemies of the gipsy moth from its native homes in Europe and Japan. The first definite printed record of the wilt disease is the one given two years later by Howard and Fiske (9). One of the tachinid flies, *Compsilura concinnata* Meig., in the various stages of its life history is especially well adapted to aid in the rapid dispersion of the disease. This imported parasite and others which are spreading rapidly may be the cause of the prodigious increase in wilt mortality since 1907.

Finally, the wilt of the tent caterpillar and that of the gipsy moth may possibly be identical, and the latter, though not previously affected, for some reason may have become susceptible to the disease of the former. This last theory does not seem very plausible, however, and ought not to be considered seriously till we have experimental proof of the identity of the wilt diseases of the tent caterpillar and gipsy moth.

DISTRIBUTION AND EPIDEMIOLOGY OF WILT

There is every reason to suppose that the wilt is distributed over the entire territory infested by the gipsy moth. In the summer of 1913 the writer personally visited places in Maine, New Hampshire, Massachusetts, and Rhode Island during the caterpillar season and found the disease to a greater or less extent in all these States. To be absolutely certain, material was always collected from the points visited and was later examined microscopically for polyhedra (p. 104). The field men of the Bureau of Entomology scattered throughout the area of infestation sent in diseased material from localities which the writer was unable to visit. In this way records of some 112 separate localities where the disease occurred, including the writer's observations, were obtained.

At present the gipsy-moth-infested area in Maine embraces about 4,850 square miles; in New Hampshire, 4,960 square miles; in Massachusetts, 4,975 square miles; and in Rhode Island, 450 square miles. Wilt, based on microscopical examinations, was found in 4 places

visited in Maine, 15 in New Hampshire, 90 in Massachusetts, and 3 in Rhode Island. That the number of infested places in Massachusetts given above exceeds those of New Hampshire is probably owing to the fact that 75 more places were studied in the former than in the latter State and that the number in New Hampshire exceeds that in Maine is due to a similar reason, for 11 more localities were visited in New Hampshire than in Maine. In short, the disease was found wherever close and continuous observations were made, with the possible exception of one or two places, but even these were doubtfully healthy.

The epidemiology of wilt is not noticeably different from that of Wipfelkrankheit, for the writer was able to confirm most of the field observations of Wahl (18), Tubeuf (14, 15), and others who have studied the nun-moth disease. When a territory becomes heavily infested, an epidemic occurs sooner or later, for these larvæ defoliate all the trees and later many congregate in masses on the trunks. Naturally when the disease breaks out in such a mass most of the caterpillars become infected, and since they are everywhere abundant and are crawling around in search of food, infected individuals rapidly spread the disease. The lack of food, which is necessarily brought on by defoliation, furthermore, causes caterpillars to lose their vitality, producing greater susceptibility to the disease. Gipsy-moth caterpillars mature in July, when it is usually very hot, and after having stripped a tract of woodland of its leaves, they are almost entirely exposed to the sun's rays. Escherich and Miyajima (4) have shown experimentally that sunlight can convert the chronic into the acute form of wilt, and one can readily become convinced of the accuracy of this observation by visiting a heavily infested, stripped piece of woodland during a hot spell. Thousands of gipsy-moth caterpillars that have died of this disease will be found hanging to limbs and tree trunks (Pl. XI). There will be an enormous reduction in the number of adult moths and consequently in the number of egg clusters, but a complete extermination does not take place, owing in part to the immunity of certain individuals (p. 124).

In a lightly infested woodland the conditions are different. Here the caterpillars are much more widely separated and an epidemic is not produced. There is sufficient food throughout the season, and the trees are never completely defoliated; hence, caterpillars can always find cool places in which to rest during the midday heat. Yet even in such a favorable locality a few caterpillars will die, but the mortality as a whole is small in proportion to the number of individuals; many apparently escape infection, and most of the next generation will escape likewise the following year, unless the increase of caterpillars produces an epidemic.

Wilt is more prevalent among the older caterpillars for the reasons given above, but younger caterpillars also die of the disease, as is shown by field observations during the season of 1913, when a few typical cases were found as early as May 27, at which time the temperature

ranged between 51° and 69° F. Of course, it has been known for a long time that first and second stage caterpillars will die of the wilt when kept in a warm laboratory under unfavorable conditions, but the foregoing observations demonstrated that sometimes these caterpillars will succumb in the field even under what seem to be most favorable conditions. Many small caterpillars die of starvation, especially on coniferous trees, and many undoubtedly meet death after exposure on a cold night; therefore no diagnosis will be valid unless made microscopically for the polyhedra described below.

In some localities studied during the summer of 1913 wilt did not appear until very late in the season, when most of the caterpillars were full grown and pupating. In an infestation near Provincetown on Cape Cod, Mass., no indications of the disease were found till the very last of July, when the caterpillars were beginning to pupate. The colonies at Provincetown are isolated from the remaining infestations in Massachusetts and it seemed likely that the disease had not spread to this locality, but visits during the latter part of the season demonstrated the existence of true cases of wilt. Provincetown faces the Atlantic, and the rather cool climate may have kept diseased individuals in a chronic condition for a long time. That is the reason wilt was not noticed earlier. This example is cited simply to show how careful one must be in pronouncing a locality healthy.

PATHOLOGY OF WILT

When a caterpillar dies of wilt, all of its tissues are in a state of disorganization. The intestine is the last internal organ to disintegrate, owing to the fact, perhaps, that it is capable of resisting the fermentative or toxic character of the virus longer than the remaining tissues. If a smear of the brown liquid from a dead caterpillar is examined microscopically with a high-power dry or oil-immersion lens, it will be found to contain, besides the elements of disorganized tissues, myriads of polyhedral bodies of various sizes. (Pl. XII, fig. 1, and text fig. 1.) The average polyhedron measures from 1 to 6μ in diameter, and the individual faces of such a single body vary also. Certain polyhedra have been found to measure $\frac{1}{2}\mu$ and less, while still others reach the size of 15μ. If there is plenty of liquid on the slide, air currents will cause the polyhedral bodies to turn over and over, so that one can obtain excellent views of all their faces. Their shape varies as much as their size, but in general the form is that of a polyhedron, with more or less rounded angles. They never assume the shape of a perfect sphere, and an actual geometric outline has never been observed, as is the case with the silkworm polyhedra, which are almost perfect octahedra. (Pl. XIII, fig. 1.) In general appearance the polyhedra of the gipsy moth are more like those of the nun moth than those of the silkworm.

The wilt polyhedra are highly refractive, and on focusing they are seen to have a denser center differentiated from a somewhat lighter peripheral mass. Sometimes within the bodies concentric layers like those of an onion are observable. Often two polyhedra are seen adhering to one another, as if in the act of dividing (Pl. XIII, fig. 2), but an actual division in a hanging-drop has never been noticed, although several preparations were kept upon the microscope stage under continual observation for more than six hours. When pressure is applied to the cover glass, the polyhedra crack very readily into a number of pieces (Pl. XIII, fig. 3–10), and often without the application of pressure the same fragmentation may be observed to occur somewhat more slowly. In the latter case a notch appears at one side of the polyhedron, which gradually lengthens into a line progressing slowly toward the other side, much like the cracking of ice. Usually before the line has completely separated the two halves other lines appear, and soon the entire polyhedron is divided into a number of pieces, which may separate or may stick together in a rosette-like fashion. At no time was anything observed to come out of the polyhedra when they cracked in this manner. If the cover glass is moved while applying a little pressure, one half of the polyhedron may sometimes be folded upon the other half without the cracks appearing, showing that it is composed of a tough substance and is not at all brittle, like inorganic crystals.

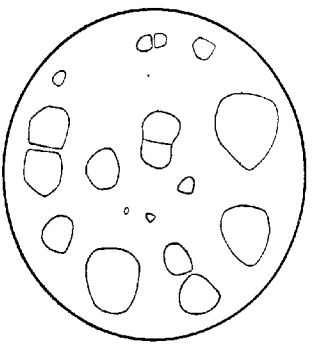

FIG. 1.—Drawing of polyhedral bodies as seen in smears of "wilted" caterpillars.

The only objects in a fresh preparation with which one could possibly confuse the polyhedra are the fat globules and urate crystals, but with a little practice these may be readily distinguished. Fat globules are perfectly spherical and are therefore unlike the polyhedral shape of the bodies in question; but, when in doubt, Sudan III was used, for in this stain the fat globules become red, while the polyhedra remain colorless. The urate crystals are often more acutely angular or are of an entirely different shape from the polyhedra and are frequently traversed by radiating lines (Pl. XIII, fig. 11–18).

Besides polyhedral bodies, fat globules, and urates, a smear from a newly "wilted" caterpillar contains cellular débris, hairs, and pigment granules. The pigment granules must not be confused with bacteria,

for many of them superficially resemble these organisms very closely. When a preparation is dried, mounted, and examined under oil, the pigment granules of the gipsy moth may easily be confused with micrococci, owing to the fact that they are usually arrayed in pairs. As a matter of fact, a smear made from a recently wilted caterpillar is almost devoid of bacteria, and in many cases none at all can be found. If bacteria are found, they have escaped into the body cavity through rupture of the intestine and bear no direct etiological relation to the disease, as will be shown later. The very minute, double, dancing granules in a fresh smear, apparently neither pigment nor bacteria, will be treated in more detail in later pages.

In fixed and stained smears a number of things can be demonstrated to advantage within the polyhedra. Fixation was accomplished either by passing the preparation through a flame or by placing it in absolute alcohol for a few minutes. The smears were then stained in Giemsa's solution for 12 hours or were stained for a shorter period with one of the following dyes: Methylene blue, trypan blue, gentian violet, carbol fuchsin, Bismarck brown, or iron hæmatoxylin. When iron hæmatoxylin was used, the preparation was first mordanted in a 4 per cent ferric-alum solution for two or three hours. Gram's method of staining, Moeller's spore stain, and Welch's capsule stain were also tried, but these were of no greater advantage than the simpler ones given above. After staining, the preparations were sometimes quickly passed through the alcohols to xylol before mounting. This not only clears everything, but dissolves away all the fat, thus increasing the transparency of the preparation. The polyhedra are very resistant to stains in general and usually color along the periphery only, unless the stain is applied for a long time. On so doing one can succeed in staining the entire polyhedron, especially after the use of some mordant like ferric alum before hæmatoxylin or anilin water before gentian violet. Steaming the preparation with a stain like carbol fuchsin has also given good results.

When properly stained, one of three conditions is obtained: First, the polyhedral bodies are uniformly stained so that nothing can be detected within them; or, second, a uniformly darker staining central mass can easily be differentiated from an almost unstained outer substance (Pl. XIII, fig. 19); or, third, many little refractive, reddish granules are seen within the polyhedra (Pl. XIII, fig. 20). I have obtained very good preparations of these three conditions, especially by the use of iron hæmatoxylin, methylene blue, and gentian violet. An actual differentiation between what might be interpreted as nuclear and cytoplasmic material within the polyhedra never occurs; therefore, in accounting for the staining reactions the writer believes that at times the polyhedra have a central granular or homogeneous substance easily distinguishable from an outer substance which is more resistant to the dyes. This varies a great deal, however, for very often the periphery of the

polyhedra takes the stain more readily than the underlying strata. From these staining reactions it becomes apparent that the polyhedra of the gipsy moth are complicated in structure, thus not differing essentially from what Bolle (1) and Prowazek (12, p. 277–281) found to be true of the silkworm polyhedra.

The polyhedra are heavier than water and consequently can be obtained in bulk by centrifuging aqueous emulsions of diseased material. By repeated washing and centrifuging, most of the fat, the cellular débris, etc., can be eliminated and the polyhedra obtained in a fairly pure stage for chemical tests. The writer has found, as did Prowazek (11) that 1 per cent of sodium hydroxid or potassium hydroxid swells the polyhedra to about double their normal size and that the same granular mass observed above again becomes visible. After a time the granular mass flows together and disappears, a sort of shadow remaining. On treating the material with dilute hydrochloric or sulphuric acid, the granular mass reappears and flows together; then the periphery of the polyhedron dissolves away, and a shadow remains, which also quickly vanishes. The wilt polyhedra do not dissolve in hot or cold water, and are insoluble in alcohol, ether, chloroform, xylol, and glycerin, but are soluble in strong acids and alkalies. They do not blacken with osmic acid and do not stain with Sudan III and therefore contain no fat. Picric acid stains them yellow, showing that they are related to the albuminoid substances.

So far nothing tangible has appeared that would enable the writer to regard the polyhedral bodies as organisms, and therefore he believes them to be reaction bodies belonging to the highly differentiated albumins—namely, the nucleoproteids. It will be shown later that the polyhedra originate in the tissue nuclei; hence, the conception of nucleoproteid reaction bodies does not seem unjustifiable. Furthermore, experiments discussed later in this article show that it is possible to infect caterpillars with material from which the polyhedra have been removed.

Although the writer has examined thousands of stained smears of wilted caterpillars, he has never observed anything which could be associated with the chlamydozoa as described in 1907 by Prowazek (11). In his latest paper, however, Prowazek (12) himself says very little about the chlamydozoa and therefore seems no longer to be greatly impressed with their etiological importance.

TECHNIQUE

Before going into a detailed consideration of the pathology of the tissues, a description of a number of the fixing and staining methods that were used may not be out of place.

The best results were obtained by the use of Giemsa's stain, Schaudinn's corrosive-sublimate fixation being used whenever this stain was employed. Hot water was saturated with corrosive sublimate and allowed to cool. Two parts of this solution to one part of absolute alcohol constituted the fixing fluid, which was used cold. The

caterpillars, if small, were either punctured or split open dorsally before being put into the solution or, if large, were cut crosswise into three or four pieces and allowed to fix for 48 hours. After using the fixing fluid, the caterpillars were immersed in 95 per cent alcohol, then in 100 per cent alcohol, and next in cedar oil, in which the material was cleared for 48 hours. The specimens were then embedded in paraffin, and sections were cut of the thickness of 2, 3, 4, or 5μ.

The process of staining and differentiation used is based upon Wolbach's modification of Giemsa's method.[1] The various processes to which the cut sections were subjected according to this method can be followed best in a numerical series.

(1) Xylol; (2) absolute alcohol; (3) 95 per cent alcohol; (4) iodin alcohol (100 c. c. of 70 per cent alcohol plus 3 or 4 c. c. of a saturated alcoholic solution of iodin); (5) 95 per cent alcohol; (6) distilled water; (7) hyposulphite of soda (0.5 per cent) in distilled water; (8) washing sections in running tap water for five minutes; (9) rinsing in distilled water; (10) staining with Giemsa's solution, changing stain twice at one-half hour intervals, leaving sections in third solution overnight (about 12 to 15 hours); (11) acetone-colophonium mixture for about one minute (this differentiates and consists of 30 gm. of colophonium to 200 c. c. of acetone); (12) acetone-xylol mixture, which stops the destaining and consists of 70 c. c. of xylol and 30 c. c. of acetone; (13) xylol; (14) mounting in thick cedar oil.

The Giemsa's solution was made from the stains of Grübler's manufacture. At first the mixture was bought all ready "made up" by local chemists, but the results were unsatisfactory and too variable to be depended upon. It was not until the writer made his own mixture that Giemsa's stain was a complete success.

The stock solution is made up as follows:

Azur II eosin	3 gm.
Azur II	0.8 gm.
Methyl alcohol (c. p.)	375 gm.
Glycerin (c. p.; Merck's, sp. gr., 1.250)	125 gm.

The staining solution is made when needed from the stock solution, as follows:

Distilled water	100 c. c.
Methyl alcohol	4 c. c.

Stain, 40 drops from an eye dropper; 0.5 per cent sodium carbonate, 2 drops from an eye dropper.

Another stain following the corrosive-sublimate fixation and giving very good results is Unna's polychrome blue, which consists of a 1 per cent aqueous solution of methylene blue to which has been added 1 gm. of sodium carbonate. This is allowed "to ripen" for one week. Following are the steps in the staining process to which the sections are subjected:

(1) 5 per cent aqueous w. g. eosin for 20 minutes; (2) Unna's polychrome blue 10 c. c. and 100 c. c. of water till the sections are a deep blue; (3) tap water; (4) differentiation in 95 per cent alcohol containing 10 per cent of resin; (5) absolute alcohol, xylol, Canada balsam.

Some good slides were obtained by fixing with Kahle's fluid and by staining with iron hæmatoxylin and orange G; but this method was not as satisfactory as another one suggested by Prof. Gary N. Calkins, in which the material is fixed for an hour in a fluid consisting of 20 per cent of glacial acetic acid and 80 per cent of saturated aqueous corrosive sublimate. The sections are mordanted for 12 hours in a 4 per cent solution of ferric alum, after which they are stained in a 0.5 per cent solution of aqueous iron hæmatoxylin for 12 to 24 hours. No counterstain is used, for by differentiating with the ferric alum the staining can be stopped at a point where both nucleus and cytoplasm are nicely colored.

[1] Wolbach, S. B. The filterable viruses. A summary. *In* Jour. Med. Research, v. 27 (n. s. v. 22), no. 1, p. 1–25, 1 fig.

PATHOLOGY OF THE TISSUES

As stated previously, owing to the fact that dead caterpillars disintegrate completely, they can not be used for sectioning; so one has to rely entirely on living, diseased material. By sectioning large numbers of caterpillars or by infecting a number of individuals and sectioning one every few days, all stages of the disease can be obtained. The writer has sectioned between 600 and 700 individuals in all stages of development, from the fully formed embryo within the egg up to the pupa. Polyhedral bodies have never been found in gipsy-moth eggs, although both apparently normal eggs and eggs that did not hatch were carefully examined. The pathological conditions in the post-embryonic stages—i. e., from the first to the sixth or seventh instars—were found to be exactly alike, showing that the pathology does not vary at different ages.

If the anterior and posterior ends of an infected caterpillar be cut off so that the alimentary canal can be pulled out easily, one will find on examination under a low-power microscope that the trachea and its finer branches have grapelike clusters of rounded bodies attached to them. Upon examination under the high-power dry or oil-immersion lens, however, one finds that the clusters are simply masses of polyhedral bodies within the nuclei of the tracheal matrix cells. (Pl. XII, fig. 2.) The nuclei of these cells seem to be among the first to be affected, for often one will have no difficulty in finding polyhedral bodies around the tracheæ, when none will be revealed by a careful search in the other tissues. Later, the polyhedral bodies appear also within the nuclei of the hypodermal, fat, and blood cells. If the pathological nuclei in their earlier stages—i. e., before the polyhedral bodies have reached their final stage of development—are carefully examined under oil, many minute violently dancing granules will be found within them. The dancing granules may be particles of degenerated chromatic or achromatic substance, but the activities are so violent even in a preparation from which all air currents have been excluded with vaseline that the writer is inclined to think that there was more than molecular motion and that he was confronted with the behavior of extremely minute micro-organisms. These granules are similar to those found in the fresh smears of dead caterpillars mentioned previously, in distinguishing between pigment and other more violently dancing particles. For reference to these bodies in stained sections, see page 110.

Stained sections show that the polyhedra originate within the nuclei of the hypodermal, fat, tracheal matrix, and blood cells. The writer has been utterly unable to find polyhedra within the nuclei of the muscles, Malpighian tubes, ganglia, or nerves. It is also interesting to note that polyhedral bodies have never been found within the nuclei of gland cells, such as setiferous cells, intestinal epithelial cells, œnocytes, salivary glands, and gonads.

The formation of the polyhedral bodies within the nuclei of the four tissues above mentioned and the visible changes taking place within these nuclei may be described as follows: The first indication of a diseased nucleus seems to consist in the flowing together of the chromatin into a lump in the middle (Pl. XIII, fig. 21). Then out of the achromatic substance the polyhedra arise as very minute individuals (Pl. XIII, fig. 22), which can be demonstrated to advantage by the hæmatoxylin method given under "Technique." By this method the polyhedra are stained dark; by the use of Giemsa's stain they are merely faintly outlined (Pl. XIII, fig. 23). At this stage Giemsa's stain also clearly shows many little granules in the nuclei (Pl. XIII, fig. 23) which are identical with the dancing granules observed in fresh preparations. They stain red and are either single or double, thus resembling tiny micrococci. These granules may adhere to the periphery of the polyhedra or may lie above, below, or in the spaces between them. The formative polyhedra themselves stain slightly along the periphery with Giemsa's stain. As the polyhedra increase in size, they become more and more refractive, do not stain at all finally, and the nucleus swells to an enormous size (Pl. XII, fig. 3; XIII, fig. 24). To obtain some idea of the comparative sizes of normal and pathological nuclei in the same tissue of the caterpillar, 12 normal and 12 pathological fat cell nuclei were measured. The normal nuclei measured between 6 and 13μ, the pathological nuclei from 7 to 29μ in diameter. The early pathological stages measured less than the later ones, and it is seen from the measurements that the late stages of the hypertrophied nuclei are more than twice as large as the largest normal nucleus. This swelling of the nucleus is due to the increase in size of the polyhedral bodies, which stretch the nuclear membrane. All the polyhedra seem to be in the same stage of development within an individual nucleus—that is, great differences in sizes between polyhedra within a single nucleus do not occur, but there are, of course, enormous variations in sizes between those of separate nuclei. The small polyhedra are somewhat rounder than larger individuals, which can be accounted for by the fact that, as the polyhedra grow, they become so closely packed within a nucleus that they press upon one another and thus the more or less polygonal shape is produced. As the polyhedra grow and become more refractive, the little red granules stained by Giemsa's stain, as well as the remains of the chromatin lump, disappear and there remains simply the nuclear membrane inclosing the polyhedra (Pl. XIII, fig. 24). Sometimes the chromatin lump remains till the nucleus disintegrates, but most frequently it disappears before this event. The nucleus swells more and more, finally the nuclear membrane ruptures, and the polyhedra escape into the body cavity (Pl. XIII, fig. 25). Thus, the polyhedra are found free in great numbers in smears of dead caterpillars.

Although these bodies are not formed within the nuclei of muscle, nerve, excretory, and glandular cells, it is not the intention to imply that no changes at all take place within these, for such is not the case. Their chromatin shows signs of degeneration, such as the flowing together into lumps, but the little reddish-staining granules were never found within them. This leads the writer to believe that the little granules are not products of nuclear disintegration—if they were, one would expect to find them within these nuclei also—but that they are of etiological significance. While, of course, they may be the vegetative stages and the polyhedra the resting stages of an unknown organism, there is nothing tangible which would substantiate the view that the polyhedra are directly related to these granules. The latter are not identical with those appearances described as being within the polyhedra (p. 106). It will be shown later in this paper that the virus passes through the pores of the Berkefeld filter, and since such a filter holds back polyhedra one might say that these have been satisfactorily eliminated and therefore are of no etiological significance; but this, it seems to the writer, is a narrow view to take of the subject. The Berkefeld filtrate revealed little dancing granules which may be identical with those observed within the tissue nuclei. Now, as before stated, these filterable granules may be the vegetative stages and the polyhedral bodies the resting stages of an organism; or the polyhedra may be a secretion of a minute organism contained within. As long as there is no evidence, however, that the polyhedral bodies are directly related to the filterable virus or to the little granules, the view that they are reaction products appeals more strongly. The virus invades the nuclei of the hypodermal, fat, tracheal matrix, and blood cells, and the polyhedral bodies arise, perhaps, as by-products of nuclear digestion and disintegration. When these four tissues disintegrate, it is an easy matter to conceive the disorganization of the remaining tissues, and, as stated previously, the intestine seems to be one of the last organs in the body to be so affected.

The questionable little granules should not be confused with the pigment granules occurring in the hypodermal cells and in the ganglia. Since the pigment granules are larger and are never found within the nuclei, unless carried there by the microtome knife, they are very easily distinguished. Furthermore, the ordinary protein bodies often occurring in the spaces of the fat body and easily demonstrated by hæmatoxylin must not be taken for polyhedral bodies. Protein bodies stain perfectly black with hæmatoxylin, are of a round or a regular shape, and are never found within the nuclei.

Polyhedra have never been found in the intestinal lumen. One would often expect to find them there, especially after artificially feeding polyhedral material to caterpillars, but this does not prove to be the case. However, their absence may be explained by the observations of

Bolle[1] and Prowazek (11). Prowazek found that pepsin hydrochloric acid dissolves the polyhedra of the silkworm, and Bolle found that the juices of silkworm intestines dissolve them likewise, so that it is not at all improbable that the gipsy-moth caterpillars digest polyhedral bodies very rapidly.

The only things found within the intestinal lumen of caterpillars are partly undigested leaf cells and occasional bacteria. The latter are sometimes found in great numbers in the intestines of caterpillars raised on abnormal food in the laboratory, but they are scarcely ever found in sections through diseased individuals taken in the field. Lettuce was frequently fed to caterpillars hatched in the laboratory in the winter, but this is unfavorable food, as cultures showed, being full of bacteria of all sorts, especially when after standing it begins to ferment. That bacteria are not etiologically related to wilt will be shown more definitely in this paper.

PATHOLOGY OF THE BLOOD

Before considering the blood of diseased gipsy-moth caterpillars, the various elements in the hæmolymph of normal individuals should be carefully distinguished. For purposes of microscopical examination a drop of blood can be best obtained by pricking one of the caterpillar's prolegs with a fine needle. In healthy animals the blood is clear; light yellow in males and greenish in females.[2]

The morphological elements or blood corpuscles are represented by two main types. Those of the first type are the ordinary round or amœboid cells, amœbocytes (Pl. XIV, fig. 1, 2). An actual pseudopod-like streaming has never been observed, but, since we find such forms as shown in Plate XIV, figure 1, with foreign bodies within them (phagocytosis), there can be little doubt as to their mode of progression. Graber's view (8) that the form of the leucocyte is due as much to the various blood sinuses as to its own individuality is not held by the writer. Graber says that the blood corpuscles are elastic, but that on their squeezing through narrow passages or sinuses this elasticity is broken down—that is, the corpuscles reach their elastic limit and are unable to reassume their natural sphericity. Hence, the various amœboid and stellate cells are due to the shape and width of the passages traversed. It is, however, generally accepted that the blood corpuscles in most insects move in an amœboid manner, thus resembling the leucocytes of man.

All of the blood corpuscles of insects possess a nucleus, and the leucocytes of the gipsy moth are no exception to this rule. The nucleus is

[1] Cited by Prowazek (11).

[2] For these interesting sexual differences in the color of caterpillar blood see the following papers:

Geyer, Kurt. Untersuchungen über die chemische Zusammensetzung der Insektenhämolymphe und ihre Bedeutung für die geschlechtliche Differenzierung. *In* Ztschr. Wiss. Zool., Bd. 105, Heft 3, p. 349-466, fig. 58, pl. 20-22. Literaturverzeichniss, p. 488-499. 1913.

Steche, O. Beobachtungen über Geschlechtsunterschiede der Hämolymph von Insektenlarven. *In* Verhandl. Deut. Zool. Gesell., Bd. 22, p. 272-281. 1912.

difficult to see in fresh preparations; but, if a little acetic acid is added or if the corpuscles are properly fixed and stained, it can be very easily demonstrated.

To the second type of corpuscle belong curious corpuscles filled with thick colorless globules (Pl. XIV, fig. 3, 4). This type is not so plentiful as the amœboid, but one often finds two or three of them to a single field. They are nearly always spherical and never emit pseudopodia. At first the writer confused these corpuscles with the pathological forms, for the colorless globules within resemble polyhedra very much at times. They are perfectly normal appearances, however, and are similar to the "mulberry corpuscles" of Forbes (5) and to the corpuscles described by Cuénot in 1891 (2). Cuénot says, in his work on the grass egger (*Gastropacha trifolii*) that the globules within the "mulberry corpuscles" color yellow with iodin and present all the various albumin reactions. He further states that they are reserve amœbocytes and that various transitional stages between them and the ordinary amœbocytes, in which the albumin (protein) globules accumulate gradually, can be detected. The "mulberry corpuscles" likewise possess a nucleus, which is surrounded by the protein bodies. This nucleus can be very easily seen by crushing the corpuscle (Pl. XIV, fig. 5).

When a gipsy-moth caterpillar becomes infected with wilt, polyhedral bodies begin to form within the nuclei of the amœbocytes in a manner very similar to their method of formation within the nuclei of the tissue cells previously described. When the polyhedra originate within the nuclei of the blood corpuscles, they have the appearance shown in Plate XIV, figure 6. The same dancing granules referred to as occurring in the nuclei of tissue cells prior to the appearance of fully formed polyhedra were also noted within the nuclei of many corpuscles. In diseased caterpillars many corpuscles are encountered which have only one polyhedron, or several, within them (Pl. XIV, figs. 7–9). Such individual or widely separated polyhedra were not formed within the nuclei of the blood cells, but were probably phagocytized by them on escaping into the hæmolymph through rupture of certain tissue nuclei. Another pathological form of corpuscle encountered very often is that shown in Plate XIV, figure 10. Here the entire cytoplasm of the cell seems to have disappeared, having been used, perhaps, as nutriment by the virus, and all that remains is the cellular membrane and a nucleus containing several polyhedra. The writer is certain that this is a pathological condition of the corpuscles, for these cytoplasmic-free elements were never found in normal animals.

As the disease progresses, more and more corpuscles become filled with polyhedra, and the number of polyhedra floating freely in the plasma increases appreciably. As stated above, these bodies escape into the blood through the disintegration of tissue nuclei, so that a large number of polyhedra in the plasma is a good indication of the final stage of the disease within the tissues.

Escherich and Miyajima (4) in their work on *Wipfelkrankheit* consider the blood of nun-moth caterpillars as an absolute and reliable index of conditions existing within the tissue nuclei. Before using caterpillars for their experiments, these authors repeatedly examined the blood for polyhedra, allowing a stated interval to elapse between successive examinations. If the last test did not reveal polyhedra within the corpuscles, the caterpillars were diagnosed as healthy; if the blood contained polyhedra, however, the animals were pronounced diseased and were discarded. Such a procedure consumes a great deal of time, and since time is an important factor in a working season that extends over only eight weeks this method was soon abandoned by the writer in his experiments on gipsy-moth caterpillars. In order to procure healthy material, a much more serviceable method, described in the section dealing with the infection experiments, was used. Nevertheless, in order to ascertain the accuracy of the blood test, the hæmolymph of a large series of caterpillars was examined, after which they were sectioned, that conditions within the tissue nuclei might be checked with the appearance in the blood. Table I gives the results of one series of experiments.

TABLE I.—*Results of blood tests and tissue examination of gipsy-moth caterpillars*[a]

Test No.	Examination of blood	Examination of tissue sections.
1	Moderate number of polyhedra in corpuscles	Nuclei filled with polyhedra.
2	....do	Do.
3	....do	Do.
4	....do	Do.
5	Few polyhedra in corpuscles	Do.
6	....do	Do.
7	Moderate number of polyhedra in corpuscles	Do.
8	Large number of polyhedra in corpuscles and many free in plasma.	Do.
9	Few polyhedra in corpuscles	Nuclei normal.
10	....do	Do.
11	No polyhedra in corpuscles	Do.
12	....do	Do.
13	Moderate number of polyhedra in corpuscles	Nuclei filled with polyhedra.
14	Large number of polyhedra in corpuscles	Do.
15	No polyhedra in corpuscles	Nuclei normal.
16	Moderate number of polyhedra in corpuscles	Nuclei filled with polyhedra.
17	Moderate number of polyhedra in corpuscles and a few out in plasma.	Do.
18	Moderate number of polyhedra in corpuscles	Do.
19	....do	Do.
20	Moderate number of polyhedra in corpuscles and a few within the plasma.	Do.
21	Large number of polyhedra in corpuscles and a few out in plasma.	Do.
22	Large number of polyhedra in corpuscles and many out in plasma.	Nuclei filled with polyhedra and many nuclei disintegrating, thus freeing polyhedra.
23	Large number of polyhedra in corpuscles	Nuclei normal.
24	Large number of polyhedra in corpuscles and many out in plasma.	Nuclei filled with polyhedra and many nuclei disintegrating, thus freeing polyhedra.

[a] The words, "few," "moderate," and "large" in Table I are simply comparative terms that are also used by Escherich and Miyajima (4). A "few polyhedra within the corpuscles" means an occasional find, possibly one or two scattered over many fields examined. By "moderate number" is meant two or three polyhedra within perhaps a third of the corpuscles in a field and represents also a few out in the plasma. Blood classified under "large number" gives a good picture of a caterpillar in the last stages of the disease, for nearly every corpuscle is beset with numerous polyhedra, and, likewise, many are floating about in the plasma.

It will be seen from Table I that the blood, in general, is a fairly reliable index of a caterpillar's condition. Still, it must be noticed that under tests 9, 10, and 23 the conditions in the blood did not represent those of the tissues. However, not a single case was found where polyhedra were in the tissues, but not in the blood. Tests 9, 10, and 23 also indicate that the blood is the first tissue to be affected. This is to be expected, since infection, as will be shown later, occurs by way of the alimentary tract. The virus is ingested with the food and possibly passes through the epithelium of the intestine into the hæmolymph, by means of which it is distributed to the other tissues.

In 1913 both Dr. Chapman and the writer were fully aware of the impracticability of the blood test and also did not consider it an accurate means of diagnosing diseases of caterpillars. After more extensive studies, however, the conclusion has been reached that the blood test, though not irreproachable, is, nevertheless, a better index than it seemed at first. If used at all, the test must be made with extreme care, but, owing to the length of time it requires, it can not be used when one is working with several hundred caterpillars.

ETIOLOGICAL INVESTIGATIONS

In 1913 the problem was again attacked in the most critical manner from the bacteriological point of view. An account of all the work undertaken will not be given, as the results were entirely negative, not essentially differing from those obtained by Jones (10). After a caterpillar has been dead for some time, bacterial invaders enter and cultures are not difficult to obtain; but the writer found, as Jones had, that, when attempts were made to isolate bacteria from caterpillars that had recently died in the field, the tubes remained sterile in most cases, and that, when a growth was obtained, the different species of bacteria isolated behaved as a non-pathogenic intestinal flora. Among all these attempts, no growth was obtained in tubes inoculated with material taken from the body cavity of living diseased animals. The media used, at varying degrees of acidity and alkalinity, included nutrient veal and beef agar and gelatin, bouillon, potato, and caterpillar soup. Anaerobic cultures were also tried, but the results were likewise negative. The bacteriological results bore a striking similarity to those of Tangl (13) and Wachtl and Kornauth (17) with *Wipfelkrankheit* and to the results of Jones (10) in his work on the gipsy moth.

After having satisfactorily eliminated the higher parasites (nematodes, protozoa, etc.) and after obtaining negative results bacteriologically, the following possibilities suggested themselves: (1) Either the polyhedral bodies themselves are parasites; or (2) the etiological factor of the wilt disease is a minute filterable organism independent of the polyhedra; or (3), if the virus is filterable, it may be genetically related to the polyhedra. The filterable-virus viewpoint seemed very promising, especially since

Prowazek (12) concluded from his experiments that it is sometimes possible to infect healthy silkworms with polyhedra-free filtrates. Since Escherich and Miyajima (4) and Glaser and Chapman (6) obtained negative results with Berkefeld filtrates and since Prowazek's filtration results (12) were rather indefinite, the experiments of the writer will be discussed at length, for the reason that their success depended entirely upon proper attention to seemingly insignificant details.

EXPERIMENTATION

Small caterpillars are unfit for experimentation; so it is necessary to wait until they are in the fourth or fifth stage before they can be used. This delay adds somewhat to the difficulty, because at those periods caterpillars are rapidly approaching pupation, and consequently the experimental period becomes shortened appreciably. Furthermore, since the weather is usually hot at this time, wilt is very prevalent in most places, and many more caterpillars are infected than earlier in the season, when the weather is cooler. All the experimental material was obtained directly from the field, as caterpillars raised in the laboratory are unhealthy and utterly worthless.

The first requisite for the experiments was to ascertain whether really healthy material could be obtained. As the lightly infested localities promised a greater number of healthy caterpillars than those heavily infested, a number of lightly infested localities were selected and collections were made, provided the wilt disease was not in evidence. The caterpillars were placed in new wooden boxes and shipped directly to the laboratory. Sometimes the disease broke out during transit; the entire lot was then killed and another collection made. If the insects seemed healthy on arrival, they were placed in autoclaved trays and submitted to a rigid physical examination for four or five days. It has been shown by Escherich and Miyajima (3, 4) and Prowazek (11) that the incubation period of the polyhedral diseases depends very intimately on external conditions; in other words, heat, cold, starvation, poor food, etc., will very soon convert a chronic into an acute case. For this reason these caterpillars were divided into lots and treated in a variety of ways: Some were starved, others were fed with leaves soaked in water for 48 hours, while still others were subjected to the direct heat of the sun. If a single caterpillar died of the wilt disease during the course of any of these treatments, the entire lot was discarded and a fresh collection made; but if all remained healthy, as often proved to be the case, the animals were thought safe to use. This method of obtaining healthy individuals may have its faults, no doubt, but its effectiveness will at once become apparent when the experiments are carefully examined.

Pasteboard boxes, 7 inches long by 5½ inches wide by 5 inches deep, with a cheesecloth lid, were used in all infection experiments. Several

of these boxes can be conveniently placed in an autoclave at one time, and the heat of the steam effectively penetrates through the pasteboard, thus assuring sterilization. After sterilization, a vacuum is produced and the boxes are taken out in just as dry and solid condition as when first put into the autoclave. Every box was autoclaved before using, for, even if new, they may become infected in some way during transit from the factory to the laboratory. After the boxes were removed from the autoclave, they were placed in rooms which had never contained wilt material, and one caterpillar was introduced into each box. In order to have conditions as uniform as possible, all caterpillars belonging to a single experiment were kept in the same room, being fed with red-oak leaves during the entire period. Red-oak leaves remain fresh much longer after being picked than those of either black oak or white oak and were chosen both for this reason and because red oak is one of the most favored food plants of the gipsy moth. To preclude the possibility of introducing the disease by means of the food, the red-oak leaves were shipped daily from a locality not infested by the gipsy moth.

SERIES 1

First-stage caterpillars, after having successfully passed their physical examination, were placed in their boxes on June 17 and were infected on June 18. Wilted caterpillars that had been collected a few hours before were ground up in a mortar with just enough sterile water added to facilitate the grinding. The material was then strained through cheesecloth, after which it amounted to 40 c. c., and was divided into two lots of 20 c. c. each. One lot was diluted with 25 times its volume, while the other was diluted with 50 times its volume of sterile water, in order to keep the pores of the Berkefeld filters free. A concentrated, unfiltered emulsion of diseased material is so full of polyhedral bodies, cellular débris, hairs, and pigment granules that it very soon plugs up a filter. Concentrated material, furthermore, is rather thick and slimy, and a film soon becomes deposited on the outside of the candle, thus withholding the virus. Both lots were then thoroughly shaken and filtered through paper filters by means of suction. This filtrate was passed through Berkefeld filters, grade N, and used for the infection experiments.

The filtrates were always controlled culturally and in most cases remained sterile, in so far as bacteria were concerned. One must, of course, be careful to autoclave the Berkefeld filters and all receptacles before using them. One or two cases where bacteria were obtained from tubes inoculated with Berkefeld filtrates could be traced to faulty technique. After shaking the filtrate well, 2 or 3 c. c. were always centrifuged and the bottom sediment examined microscopically for polyhedra; but these were never found, and nothing could be observed, even

in a dark field, except minute dancing granules, which may be identical with those mentioned under "Pathology of wilt" as having been observed in diseased tissue.

In the first experiment 10 caterpillars were fed with the 1 to 25 dilution passed through a Berkefeld filter, and 10 were fed with the 1 to 50 dilution passed through another Berkefeld filter. Twenty controls accompanied this series.

Ten caterpillars were fed simply with the unfiltered 1 to 25 dilution, while 10 were fed with the 1 to 50 unfiltered dilution. Fifteen controls accompanied this series.

The controls were treated in exactly the same manner as the other individuals, except that they were fed with material that had been sterilized by autoclaving. The infectious material was administered to the caterpillars in two ways: Either some of the liquid was sucked up in a sterile eye dropper, by means of which a drop was placed over the caterpillar's mouth, or the oak leaves were submerged in the material before being placed in the trays. The first method is advantageous, for the reason that a large quantity of the virus can be administered. The caterpillar is held upside down and a drop is placed directly over its mandibles. These and the maxillæ soon begin to move, and gradually the drop disappears. If caterpillars are to be infected in this manner, it is necessary to avoid the period directly preceding a molt, as they will not drink until this process has been completed. Furthermore, if caterpillars have eaten shortly before one wishes to infect them, it is difficult to make them drink; consequently the animals were usually starved for a few hours before infection. The second method is also good, and has the advantage of not consuming as much time as the first. One can be certain whether or not they have eaten by examining the old, infected leaves before replacing them with fresh foliage. In the first experiment both methods were used, and since none died up to June 25, the insects were infected again on that date in exactly the same manner as before.

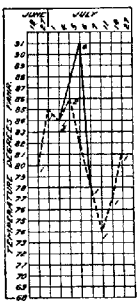

FIG. 2.—Curve showing the mortality among 20 gipsy-moth caterpillars fed with the wilt virus after filtration through the Berkefeld filter.

In order to determine the effect of temperature on the incubation period,[1] it was recorded daily at noon.

Figure 2 shows the mortality of the 20 caterpillars fed with the Berkefeld filtrate. The ordinate represents the range of temperature between June 18, when the experiment was begun, and July 27, when it was concluded. In order to avoid unnecessary space, the abscissa represents the day when the experiment was begun, the day on which caterpillars died, and the day on which the experiment was concluded.

[1] The term "incubation period" is used here as the time elapsing between infection and death.

The solid line represents the plot of the deaths from the wilt and the broken line deaths due to another cause. The number of daily deaths is inserted in the squares. When caterpillars died of the wilt disease on a certain day and others succumbed to other causes on the same day, a fraction is used, the numerator representing the number of deaths from the wilt and the denominator the other deaths.

The interpretation of the curve follows: The experiment was begun on June 18; on June 27 one caterpillar died of the "other cause" at 79° F.; on July 1, at 84°, another caterpillar died from the same cause; on July 4, at 83°, one died of the wilt disease and two died of the "other cause;" on July 5, at 85°, two died of the "other cause;" on July 6, at 90°, four died of the wilt; on July 7, at 77°, one died of the wilt; on July 11, at 73°, one died of the "other cause;" on July 14, at 76°, one died of the "other cause;" on July 27, the day the experiment ended, at 80°, one died of the "other cause."

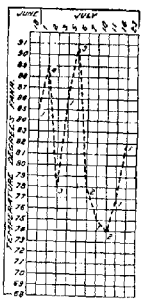

FIG. 3.—Curve showing the mortality among the 20 control gipsy-moth caterpillars. Compare with figure 2.

Summing up the results of this experiment, six deaths were caused by wilt, nine deaths were due to the "other cause," one caterpillar escaped during the course of the experiment, and four female moths emerged. Just as soon as a caterpillar died, it was examined for polyhedra, and no death was recorded as having been caused by wilt unless these bodies were in evidence. It is certain that the deaths recorded by the broken line were not due to wilt, because the animals were not flaccid and did not disintegrate on handling; on the contrary, their skin was very elastic and tough, and smears never revealed polyhedral bodies. Many round crystals with radiating lines (Pl. XIV, fig. 11, 12), however, were found. These, the writer believes, are a sign of some metabolic disturbance, and the deaths may have been caused by excessive heat coupled with dryness. Laboratory conditions are poor at best, and the food plants in the trays dried out rapidly, although replaced very often by fresh foliage. Thus, it seems that these deaths from another cause are simply a laboratory occurrence, for nothing similar in the field has ever been discovered. The first series of experiments was performed in an excessively hot room below the roof, and, while not a single control insect died of wilt, nearly all the caterpillars succumbed to this general physiological disturbance.

Attempts were made to isolate bacteria from caterpillars which died of wilt in the infection experiments, but in nearly all cases the tubes so inoculated remained sterile. This procedure always gave a good check against the platings made from the infectious material before it was used.

Figure 3 shows the mortality of the 20 controls which accompanied the 20 caterpillars fed with the Berkefeld filtrate. Of these, 19 died on

account of the physiological disturbance and 1 female moth emerged. Figure 4 gives the deaths among the 20 caterpillars fed with the unfiltered material. Of these 17 died of wilt, and 3 of the "other cause." Figure 5 shows the deaths among the 15 control insects that accompanied the caterpillars fed with the unfiltered virus. All 15 died of the "other cause." Table II gives the results of this series of experiments.

TABLE II.—*Mortality among gipsy-moth caterpillars in laboratory experiments (series 1)*

Number of caterpillars.	Treatment.	Died of wilt.	Died of "other cause."	Lived.
20	Berkefeld filtrate	6	9	[a]4 females.
20	Control	None.	19	1 female.
20	Unfiltered virus	17	3	None.
15	Control	None.	15	Do.

[a] Escaped.

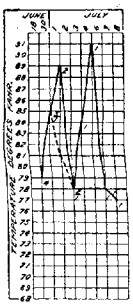

FIG. 4.—Curve showing the mortality among 20 gipsy-moth caterpillars fed with unfiltered wilt virus.

It will be seen that there are almost three times as many deaths from the unfiltered virus as from the Berkefeld filtrate. This shows that the wilt virus is filterable, but with difficulty. Although it would have been much more encouraging to have obtained moths from all the controls, still it is very significant that not a single control insect died of wilt. Noteworthy, also, are the four female moths obtained from the animals fed with the Berkefeld filtrate.

No difference was noted in the rate of deaths between caterpillars fed with the 1 to 25 dilution and those fed with the 1 to 50 dilution.

While the caterpillars in the foregoing experiments were those used in the actual experiments in this laboratory, there were, nevertheless, other caterpillars in trays in the same room. These were held in reserve as stock, but, since not a single one of them died of wilt, they may be regarded also as controls against the experiments, for they were all placed in the trays on the same day the tests were begun (June 18). They were not all the same age, however, and, hence, can not be called controls in the strictest sense of the word.

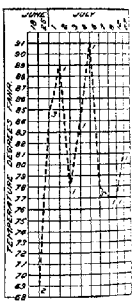

FIG. 5.—Curve showing the mortality among the 15 control gipsy-moth caterpillars. Compare with figure 4.

Figure 6 gives the mortality among 15 second-stage and third-stage caterpillars. Three were placed in each tray, and all 15 died of the "other cause."

Figure 7 represents the deaths among 26 fourth-stage caterpillars obtained from Providence, R. I., in a lightly infested point which was supposed to be entirely healthy. The disease had not appeared in the field at the time the collection was made, and although all were kept in one tray, not a single caterpillar died of wilt in the laboratory. Twenty-five died of the "other cause" and one male moth emerged.

Figure 8 gives the mortality among 8 fourth-stage caterpillars obtained from Providence. Each caterpillar was placed in a separate tray and the entire eight were starved until they died. The post-mortem appearances were so similar externally to the deaths from the "other cause" that they are represented by a broken line.

Figure 9 represents the deaths among 16 fifth-stage gipsy-moth caterpillars kept in one tray. These were from the same collection as those used in the experiments. Not one case of wilt appeared, but they all died of the "other cause."

Figure 10 shows the deaths among 19 fifth-stage caterpillars, also from the same lot as those used in the experiments.

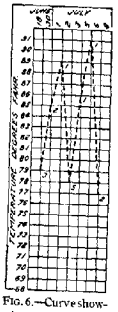

FIG. 6.—Curve showing the mortality among 15 control second-stage and third-stage gipsy-moth caterpillars.

From figures 2 and 4 it is evident that the deaths from the wilt disease all occurred during days when the temperatures were high. Taking the midway point between 79° and 80° F. in the temperature range 68° to 91°, it is found that 14 deaths occurred at and above 80° against 9 deaths below that temperature. There seems to be no definite correlation between temperature and the deaths from the "other cause."

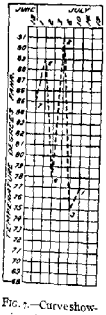

FIG. 7.—Curve showing the mortality among 26 "control" fourth-stage gipsy-moth caterpillars from Providence, R. I.

SERIES 2

The second series of experiments was begun on July 3. Last-stage caterpillars were used. Material strained through cheesecloth with sterile water to equal 50 c. c. was diluted with 20 times its volume of sterile water, then shaken, and filtered through paper filters. A portion of this filtrate was used as it was, part was filtered through the Berkefeld candles, and another portion was sterilized for the controls. The infections were repeated on July 6, and another dose was administered on July 13 to all caterpillars that had not pupated. These experiments were performed in a room some distance from the one in which the first were carried on.

Figure 11 represents the mortality among 50 caterpillars fed with the Berkefeld filtrate. Wilt caused the deaths of 13 in the caterpillar stage and of 1 in the pupal stage; 3 died of the "other cause";

10 females and 23 males emerged. Figure 12 gives the deaths among 25 caterpillars fed with the unfiltered virus. Twelve died of wilt while caterpillars and four after pupating; three died of the "other cause;" and six male moths emerged. Figure 13 shows the mortality among 25 controls accompanying the series. Three died of wilt, seven died of the "other cause," and twelve males and three females emerged. Table III gives the results of series 2.

TABLE III.—*Mortality among gipsy-moth caterpillars in laboratory experiments (series 2)*

Number of caterpillars.	Treatment.	Died of wilt.	Died of "other cause."	Lived.
50	Berkefeld filtrate	14	3	23 males. 10 females.
25	Unfiltered virus	16	3	6 males.
25	Control	3	7	12 males. 3 females.

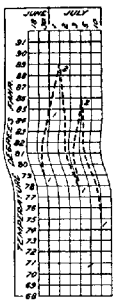

FIG. 8.—Curve showing the mortality among 8 "control" fourth-stage gipsy-moth caterpillars from Providence, R. I.

It will again be noticed that more caterpillars died when fed with the unfiltered material than when fed with the Berkefeld filtrate. If there had been as many caterpillars in the experiments with the unfiltered material as in the Berkefeld-filtrate experiment, the number of deaths from wilt in the former would probably have been more than twice as great as in the latter. Three deaths from wilt are recorded in the controls, but these caterpillars had probably contracted the disease before they were collected, as every possible precaution was taken to avoid contamination. The person who fed the infected individuals was not permitted to feed the controls and whenever a caterpillar died and had to be handled, the hands of the assistant were thoroughly washed in 95 per cent alcohol before being again allowed to handle another tray. In this series of experiments there seems to be no correlation between high temperatures and deaths due to wilt. Taking the middle of the temperature range in the entire series, there are 31 deaths below 79.5° against 2 deaths above 79.5° F. Humidity might prove to be a more important or at least an equally important factor as temperature in determining the progress of the disease; but, since no record was kept of the humidity, the writer is unable to speak with any conviction on this matter.

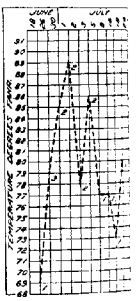

FIG. 9.—Curve showing the mortality among 25 "control" fifth-stage gipsy-moth caterpillars.

SERIES 3

Last-stage caterpillars were used in the third series of experiments, which was begun on July 14. These animals were obtained from a locality in which a tachinid, *Compsilura concinnata*, abounded. Later, during the course of the experiments, quite a large number of these caterpillars succumbed to parasitism by this species.

The wilted material was strained through cheesecloth to equal 50 c. c. This was diluted with ten times its volume of sterile water and filtered through a paper filter. This filtrate was again diluted ten times and a portion was used without further treatment while the rest was passed through Berkefeld candles.

Figure 14 represents the mortality among 40 caterpillars fed with the Berkefeld filtrate. Eight caterpillars died of wilt, seven of the "other cause," twenty succumbed to tachinid parasitism, and five male moths emerged.

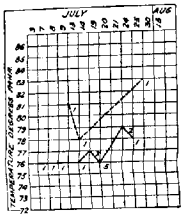

FIG. 11.—Curve showing the mortality among 50 gipsy-moth caterpillars fed with wilt virus after filtration through the Berkefeld filter.

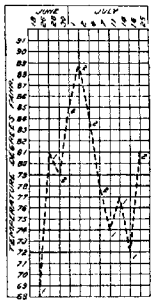

FIG. 10.—Curve showing the mortality among 19 "control" fifth-stage gipsy-moth caterpillars.

Figure 15 gives the mortality among 40 caterpillars fed with unfiltered material. Fifteen died of the wilt, four died of the "other cause," one escaped, eleven succumbed to tachinid parasitism, and nine male moths emerged.

Figure 16 shows the deaths among 20 control insects. One died of wilt, nine died of the "other cause," one succumbed to tachinid parasitism, and 5 male and 4 female moths emerged. The mortality for series 3 is given in Table IV.

TABLE IV.—*Mortality among gipsy-moth caterpillars in laboratory experiments (series 3)*

Number of caterpillars.	Treatment.	Died of wilt.	Died of "other cause."	Died of tachinid parasitism.	Lived.
40	Berkefeld filtrate	8	7	20	5 males.
40	Unfiltered virus	15	4	11	[a] 9 males.
20	Control	1	9	1	5 males. 4 females.

[a] 1 escaped.

Again, the number of caterpillars which died after feeding on the unfiltered material was almost twice as great as the number that died after feeding on the Berkefeld filtrate.

For the sake of uniformity the range of temperature is considered as extending from 71° to 82° F. Naturally the ranges of temperature are not always the same, for one experiment might extend over a greater number of days than another, thus perhaps including a few temperatures different from those in the latter experiment. Taking the midway point in the temperature range as 77°, the number of wilt deaths occurring at and above 77° is found to be 14, while there are 10 occurring below that temperature. There seems to be in this third series of experiments the same correlation between high temperature and wilt as in the first series.

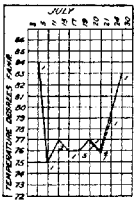

FIG. 12.—Curve showing the mortality among 25 gipsy-moth caterpillars fed with unfiltered wilt virus.

DISCUSSION OF ETIOLOGICAL EXPERIMENTS

In the three series of experiments, out of the entire number of controls, comprising 78 caterpillars, only 4 died of the wilt disease. This is equivalent to about 2.25 per cent, almost a negligible error when compared with about 39 per cent mortality in the infected animals.

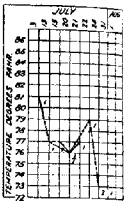

FIG. 13.—Curve showing the mortality among 25 control gipsy-moth caterpillars. Compare with figure 12.

It will be seen, on examining the diagrams, that the wilt incubation period varies considerably—from 2 to 27 days. The differences in the individual constitutions of separate caterpillars undoubtedly account for much of this variation, and slight differences in the amount of the infectious material ingested or in the virulence of the virus are important also; but such factors as heat, humidity, and food play a rôle in determining the length of the period of incubation.

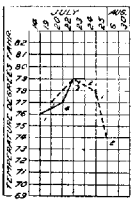

FIG. 14.—Curve showing the mortality among 40 gipsy-moth caterpillars fed with wilt virus after filtration through Berkefeld filter.

In spite of apparent infection, a number of moths emerged in each series of experiments. Out of 110 caterpillars fed with the Berkefeld filtrate, 42 adults emerged, while out of 85 individuals fed with the unfiltered material 15 imagoes were obtained. All individuals used in the infection experiments really partook of the material administered, so one can not very well account for the moths on the basis that the caterpillars escaped infection. It is possible that a genetic immunity towards wilt exists among certain members of the gipsy-moth race and that others can also be actively immunized with sublethal doses of fully virulent material.

How can we, however, account for the numerical difference existing between the moths obtained from the Berkefeld-filtrate infections and those obtained from the unfiltered-virus infections? In the experiments with the former, 38 per cent transformed as compared with 18 per cent in the latter case. As the experiments show, the virus is filterable, but with difficulty; consequently naturally unfiltered material would be more deadly than filtered material, for the reason that the former contains a greater number of micro-organisms than the latter, and caterpillars in order to contract wilt would have to ingest more of the Berkefeld filtrate than of the unfiltered virus so as to obtain the lethal dose. This will account for the greater number of moths obtained in the Berkefeld experiments as compared with those obtained in the other infections. In the case of Berkefeld infections the virus was less concentrated and more caterpillars escaped eating the lethal dose, thus perhaps acquiring an immunity toward a second infection, for it must be remembered that most of the individuals were infected a number of times.

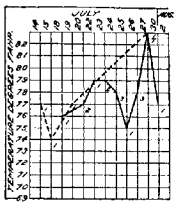

FIG. 15.—Curve showing the mortality among 40 gipsy-moth caterpillars fed with unfiltered wilt virus.

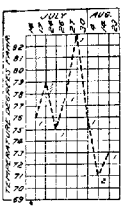

FIG. 16.—Curve showing the mortality among 20 control gipsy-moth caterpillars.

All the experimental males and females were mated and eggs were obtained which, it is hoped, will hatch so that they can be used the coming season (1914), in order to determine whether this apparent immunity is racial or merely acquired. If racial, some interesting Mendelian ratios may be obtained; if acquired, the whole of the next generation will probably be susceptible to the disease, unless certain of its members become actively immunized. If racial or acquired immunity does not exist among certain individuals of the gipsy moth, it is difficult to understand how any of these insects escape death under certain conditions in the field. The writer has often seen dozens of caterpillars congregating on trees under burlap and has seen them dying of wilt in great numbers in such places; yet, in spite of the disintegrating bodies flowing out over other individuals in the immediate proximity, many will escape death and transform.

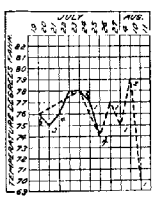

FIG. 17.—Curve showing the mortality among 50 gipsy-moth caterpillars under conditions approximating those in the field.

To repeat this field observation in the laboratory, 50 caterpillars were gathered from a locality where wilt had been raging for several weeks. The entire lot was placed in a single tray so that the dying individuals

could constantly soil the food and infect the living ones. Figure 17 gives the mortality among these insects. Twenty-one caterpillars died of the wilt disease, ten of the "other cause," eleven of tachinid parasitism, and five male and three female moths emerged. The experiment covered a period of 32 days, an ample length of time for every individual in the tray to become infected; yet in spite of the 21 deaths from wilt, 8 moths were obtained.

SUMMARY

(1) The wilt of gipsy-moth caterpillars is a true infectious disease that is distributed over the entire territory infested by the gipsy moth.

(2) Epidemics of the disease occur only in localities heavily infested by the gipsy moth.

(3) Climatic conditions appear to bear an important relation to wilt in the field.

(4) The disease is more prevalent among older than among younger caterpillars, but small caterpillars also die of it in the field.

(5) No diagnosis of wilt is valid unless polyhedra are demonstrated microscopically.

(6) There is no account of the occurrence of wilt in America prior to 1900.

(7) Minute dancing granules may be observed in wet smears.

(8) Polyhedra are probably reaction bodies belonging to the highly differentiated albumins, the nucleoproteids.

(9) The pathology of wilt does not vary with the age of the caterpillars.

(10) The polyhedra originate in the nuclei of the tracheal matrix, hypodermal, fat, and blood cells.

(11) The nuclei of the tracheal matrix and blood cells seem to be the first tissue nuclei affected.

(12) Many minute violently dancing granules are found in the pathological nuclei of fresh tissue.

(13) Giemsa's stain demonstrates many little granules in the nuclei of diseased tissue sections.

(14) The alimentary canal seems to be the last organ in the body to disintegrate.

(15) Two types of blood corpuscles exist in normal hæmolymph.

(16) Two types of pathological blood corpuscles exist in diseased caterpillars.

(17) The blood is a fairly reliable index of a caterpillar's condition.

(18) The blood test is impracticable for large experimental series.

(19) Bacteria are not etiologically related to wilt.

(20) The virus of wilt is filterable with difficulty.

(21) Such a filtrate is free from bacteria and polyhedral bodies.

(22) Caterpillars that have died from infection with filtered virus are flaccid, completely disintegrated, and full of polyhedra.

(23) Minute dancing granules were observed in the Berkefeld filtrate. These may be identical with certain granules observed in smears and tissue nuclei (sub. 7, 12, and 13) and may be etiologically significant.

(24) The incubation period of wilt varies, and temperature at times seems to bear an important relation to this variation.

(25) A large number of caterpillars used in the experiments died of disturbances in their normal physiological activities.

(26) The success of wilt infection experiments is absolutely dependent upon attention to seemingly insignificant details.

(27) Genetic immunity of certain individuals is probable.

(28) Active immunization with sublethal doses is possible.

(29) The polyhedral bodies may be stages of the filterable virus, but as yet no evidence to substantiate this view has been produced.

(30) Infection naturally takes place through the mouth by means of the food.

(31) Some of the imported parasites may be important factors in aiding the dispersion of the wilt disease.

(32) Although probable, there is no definite evidence as yet that wilt is transmitted from one generation to another.

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PLATE XI

"Wilted" gipsy-moth caterpillars hanging to a tree trunk.

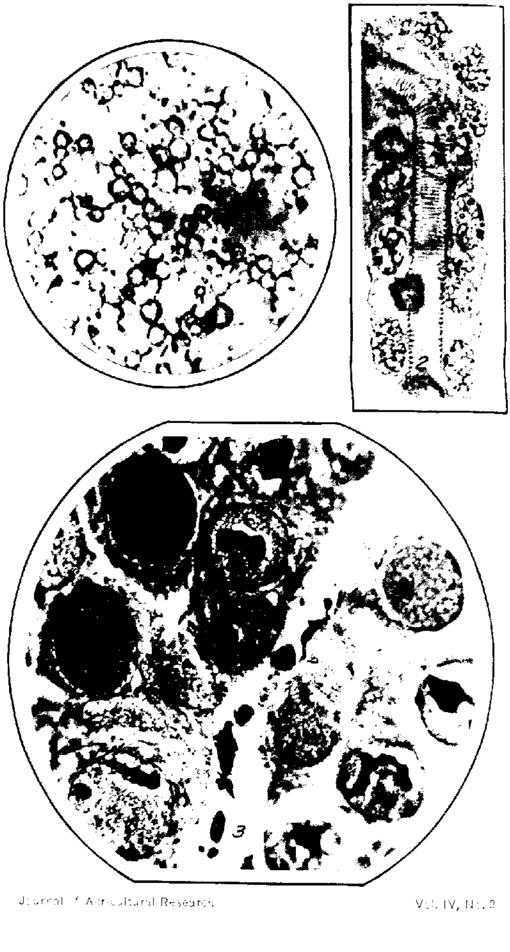


PLATE XII

Fig. 1.—Photomicrograph of a smear from a "wilted" gipsy-moth caterpillar.

Fig. 2.—Photomicrograph of polyhedra clustering around a tracheal tube of a gipsy-moth caterpillar. × 750.

Fig. 3.—Photomicrograph showing various stages during the formation of polyhedra in tissue nuclei of a gipsy-moth caterpillar. × 720.

PLATE XIII

Fig. 1.—A silkworm polyhedron, after Prowazek.

Fig. 2.—Two gipsy-moth caterpillar polyhedra adhering to each other.

Fig. 3 to 10.—Polyhedra of gipsy-moth caterpillar cracking to pieces.

Fig. 11 to 18.—Urate crystals of a gipsy-moth caterpillar.

Fig. 19.—Polyhedron of a gipsy-moth caterpillar stained, showing a dark central mass.

Fig. 20.—Polyhedron of a gipsy-moth caterpillar stained, showing refractive granules.

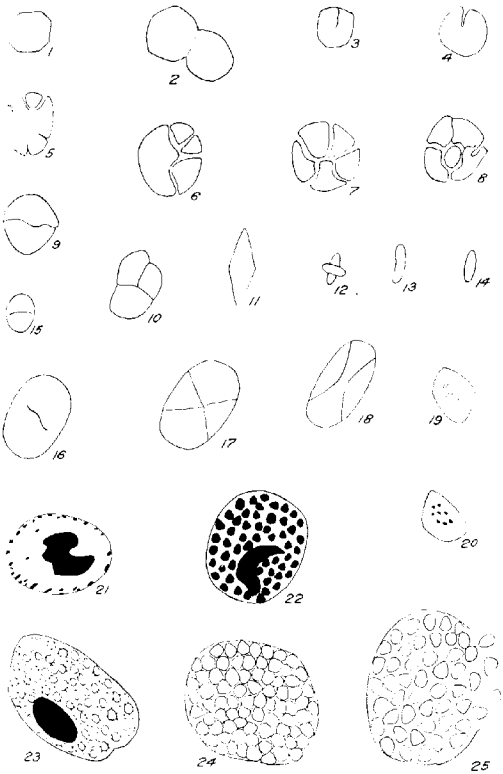
Fig. 21.—Chromatin lump in middle of pathological nucleus of a gipsy-moth caterpillar. × 950.

Fig. 22.—Iron hæmatoxylin showing stained polyhedra of a gipsy-moth caterpillar in a nucleus. × 950.

Fig. 23.—Giemsa's stain, showing unstained polyhedra of a gipsy-moth caterpillar in a nucleus and little granules. × 950.

Fig. 24.—Fully formed polyhedra of a gipsy-moth caterpillar in a nucleus. × 950.

Fig. 25.—Nuclear membrane rupturing and allowing polyhedra of a gipsy-moth caterpillar to escape. × 950.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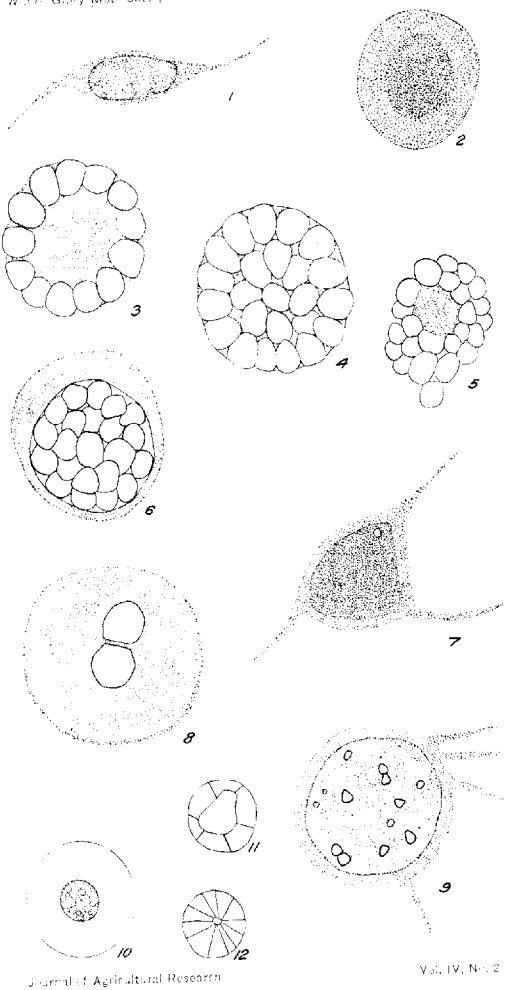
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PLATE XIV

Fig. 1 and 2.—Normal blood corpuscles of the gipsy-moth caterpillar.

Fig. 3 and 4.—"Mulberry" corpuscles of the gipsy-moth caterpillar.

Fig. 5.—"Mulberry" corpuscle of the gipsy-moth caterpillar crushed, showing nucleus.

Fig. 6.—Blood corpuscle of the gipsy-moth caterpillar, showing nucleus filled with polyhedra.

Fig. 7 to 9.—Blood corpuscles of the gipsy-moth caterpillar with phagocytized polyhedra.

Fig. 10.—Cytoplasmic-free pathological blood corpuscles of the gipsy-moth caterpillar.

Fig. 11 and 12.—Crystals found in gipsy-moth caterpillars that died of the "other cause."

EFFECT OF TEMPERATURE ON GERMINATION AND GROWTH OF THE COMMON POTATO-SCAB ORGANISM

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INTRODUCTION

The causal organism of the common potato scab which has been known to phytopathologists since 1892 as *Oospora scabies* Thaxter was recently pronounced by Lutman and Cunningham[1] as identical with *Actinomyces chromogenus* Gasperini, which was described in 1891. The writer's studies were conducted upon several strongly pathogenic strains isolated from diseased specimens received from Maine, Vermont, and Wisconsin.[2]

All these strains fruit abundantly on the so-called Thaxter's potato agar. The gray film which almost invariably occurs on the scabby spots of naturally or artificially infected tubers when first removed from the soil is made up of the same elements which constitute the fruiting stage in artificial cultures. These elements, called "gonidia," are short, cylindrical segments of aerial filaments and when mature—i. e., when the aerial growth turns from white to dark gray—were employed in making the germination studies here described. They are 1.5 to 2μ long and 0.8 to 1μ broad, with truncate ends. These bodies, after having been sown in agar and shortly before germination, become somewhat broader and rounder, sometimes oval or nearly spherical. Germ tubes may be produced at either or both ends.

EXPERIMENTAL METHODS EMPLOYED

In making the germination tests the ordinary agar hanging-block used in studying the growth of bacteria was employed. A straight transfer needle was rubbed against the surface growth of cultures bearing mature gonidia and then gently drawn across the surface of solidified agar in Petri dishes. The agar blocks for germination studies were then removed from along this inoculated streak and mounted in moist cells

[1] Lutman, B. F., and Cunningham, G. C. Potato scab. Vt. Agr. Exp. Sta. Bul. 184, 64 p., 7 fig., 12 pl. 1914.

[2] The following method was used to obtain pure cultures from these and numerous other specimens: Both the operator's hands and the diseased tuber are thoroughly washed. Then the latter is rinsed in hydrogen peroxid and dried with sterilized absorbent paper. Next the corky covering of a scabby spot is lifted off by inserting the point of a flamed scalpel under one side of it. The layer of parenchyma underneath is greenish yellow in color, owing to the action of the parasite. The discolored area thus exposed is then gently scraped with a flamed knife and a small quantity (about 1 c. c.) of the pulp transferred to tubes containing 2 or 3 c. c. of sterilized, distilled water. One or more 2 mm. loops of this dilution are transferred to tubes containing 10 c. c. of melted beef agar and the plates poured in the usual way.

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in the usual way. Beef-extract agar, without salt,[1] was found to be the most satisfactory medium for the purpose.

Immediately after the hanging-block cultures were made, the slides bearing them were placed in incubators running at the requisite temperatures. They were not removed therefrom except for a short time at the close of each hour for examination with the microscope. To avoid inaccuracies, due to possible variations in temperature in different parts of the incubator chambers, care was taken to see that the temperatures recorded were those in the immediate vicinity of the preparations studied.

THERMAL EFFECT ON GERMINATION

The maximum temperature for growth is apparently a little below 41° C., although occasionally slight evidence of the beginning of germination of gonidia was observed at this point. Germination was most rapid between 35° and 40.5°, and little or no differences were noted in various trials between these limits. At the temperatures mentioned the first evidence of growth was observed at the end of three hours, and below this the time gradually increases: 5 hours are necessary at 30°, 8 hours at 25°, 11 hours at 20°, 18 hours at 15°, and 2 days at 10° C. (fig. 1). The largest percentage of germination is usually secured at from 30° to 37° C. Unevenness in germination is evident at 25°, and from this point down it becomes more and more apparent until it is especially pronounced at 10° C. Plate XV, figures 1 and 2, illustrates the characteristic appearance of the germinating gonidia at 35° C.

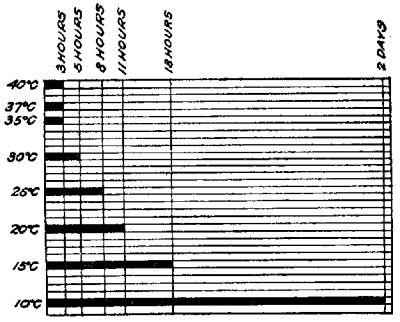


FIG. 1.—Chart showing the relation of temperature to time of germination.

No attempt was made to determine the exact minimum temperature for germination, but some previous unpublished studies of the writer indicate that it lies somewhere near 5° C. Twenty test-tube cultures, ten in beef broth and ten on potato cylinders, immediately after inoculation with material containing gonidia were placed in a refrigerator where the temperature varied from 5° to 7° C. Only a little growth was noted

[1] A modification of the formula given by F. D. Chester. (Chester, F. D. Manual of Determinative Bacteriology. p. 28. New York, 1901.)

in a few tubes at the end of one month. A few of the remaining cultures grew when taken to the laboratory, but the rest were dead.

Exposure to cold weather, several degrees below zero centigrade, does not always kill the parasite. During February and March, 1913, many test-tube cultures were exposed immediately after inoculation to freezing at outdoor temperatures and then again taken to the laboratory. The exposure in no case was longer than one week. In no instance were the organisms killed in tubes containing cooked potato cylinders, but in some cases with beef-broth cultures an exposure of five days was fatal when on some nights the thermometer registered as low as $-29°$ C.

RAPIDITY AND VIGOR OF GROWTH

Temperatures between 35° and 40° C. are most conducive to rapid germination. They are decidedly less favorable for the further development of the organism, except that at 35° the growth for the first day was more rapid than at any other temperature tested. No colonies visible to the unaided eye appear in cultures at 39.5°, and growth at this temperature practically ceases within one week. On the other hand, growth is very much retarded and slow below 20° C. Table I shows the comparative rates of germination and growth in cultures at various temperatures and at different intervals within one week.

TABLE I.—*Comparative rates of germination and growth of the common potato-scab organism at various temperatures and at different intervals*

Temperature.	Growth.						
	3 hours.	5 hours.	8 hours.	11 hours.	18 hours.	2 days.	1 week.
°C.							
40	Germination begins.	Threads 2 to 3μ	Very slight progress.	Very slight progress.	Very slight progress.	Threads 5 to 10μ.	Threads 15 to 20μ.
37	.....Do.....	Threads 5 to 8μ.	Threads 8 to 14μ.	Threads 20 to 22μ.	A network of curled threads.	Small colonies formed.	Feeble colonies of curled threads.
35	.....Do.....	Threads 5 to 14μ.	Threads 15 to 20μ.	Threads up to 70μ.	A complete network.	Colonies formed.	More or less complete growth along the line of inoculation.
30	.....	Germination begins.	Threads 5 to 8μ.	Threads 25 to 30μ.	A network.....	.....do.....	Do.
25	.....	.....	Germination begins.	Threads 11 to 22μ.	.....do.....	.....do.....	Do.
20	.....	.....	.....	Germination begins.	Threads up to 30μ.	Formation of colonies.	Do.
15	.....	.....	.....	.....	Germination begins.	A network..	Formation of colonies.
10	.....	.....	.....	.....	.....	Germination begins.	Threads 27 to 30μ.

Observations upon cultures for longer periods, two to four weeks, indicated that the optimum temperatures for maximum growth are from 25° to 30°, with practically no difference between. The total growth produced was less above and below these points. While it was still proceeding normally but at a slower rate at the lower temperatures, at 35° it was not only less but appeared to have reached its end.

The discoloration of the medium which is very characteristic of cultures of this parasite was faint or absent at and above 35° and quite intense at and below 30° C.

INVOLUTION FORMS

High temperature is considered one of the factors influencing the production of degeneration forms of bacteria,[1] but this is apparently not the case with the potato-scab organism. While the individual filaments, which at lower temperatures are long and more or less curved, appear very short and curled at 37° C., and especially so at 39° and 40°, the writer does not consider them strictly involution forms. The gonidia also, can not be considered as involution forms, since morphologically the same bodies occur normally upon scab spots on potato tubers and apparently serve as fruiting organs.

However, such abnormal growths may be produced by certain kinds of culture media. The writer has observed some very interesting involution forms which constantly appear at all temperatures when the scab organism is grown upon a synthetic agar that is much used in this laboratory for the cultivation of fungi.[2]

On this medium germination and growth proceeds normally at first, but after two days, if incubated at 35° to 37°, which, as has already been pointed out, are within the range of most favorable temperatures for germination and early growth, the threads become distorted and swollen at various places, both at the tips and in the middle. Sometimes even the gonidia themselves become abnormally enlarged at or before germination. At the end of a month the entire growth will consist of swollen, club-shaped, oval, or spherical segments of various sizes (Pl. XV, fig. 3 and 4). Not infrequently these abnormalities reach 4μ in diameter. The consistency of the growth thus produced is soft and somewhat slimy instead of being tough and hard, as is usually the case. By leaving out one of the ingredients of the medium at a time it was

[1] Migula, Walter. System der Bakterien . . . Bd. 1, p. 52–53. Jena, 1897.

[2] This synthetic agar was prepared according to the formula given by Darwin and Acton (Darwin, Francis, and Acton, E. H. Practical Physiology of Plants. ed. 3, p. 68. Cambridge, 1909) and consists of—

	Gm.		*Gm.*
Dextrose	50	Magnesium sulphate	2.5
Peptone	20	Potassium monophosphate	2.5
Ammonium nitrate	10	Calcium chlorid	0.1
Potassium nitrate	5	Distilled water	1,000

found that no such involution forms were produced when the potassium monophosphate alone was excluded, but they invariably appeared if it was present.[1]

SUMMARY

(1) Temperatures from 35° to 40° C. are most favorable for the germination of the gonidia of the potato-scab organism. They are unfavorable for long-continued growth, although at 35° a stimulating effect was produced at first.

(2) The maximum temperature for growth is about 40.5°, the optimum 25° to 30°, and the minimum about 5° C.

(3) Involution forms are produced, but not as the result of temperature conditions. They appeared abundantly when 0.25 per cent of potassium monophosphate was included in a synthetic culture medium.

[1] Münter observed similar involution forms in pure cultures of certain soil-inhabiting Actinomyces, but on a somewhat different synthetic medium than that used by the writer. Among the species studied by Münter *Actinomyces chromogenes* is mentioned. (Münter, F. Über Actinomyceten des Bodens. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 36, No. 15/18, p. 380–381. 1913.)

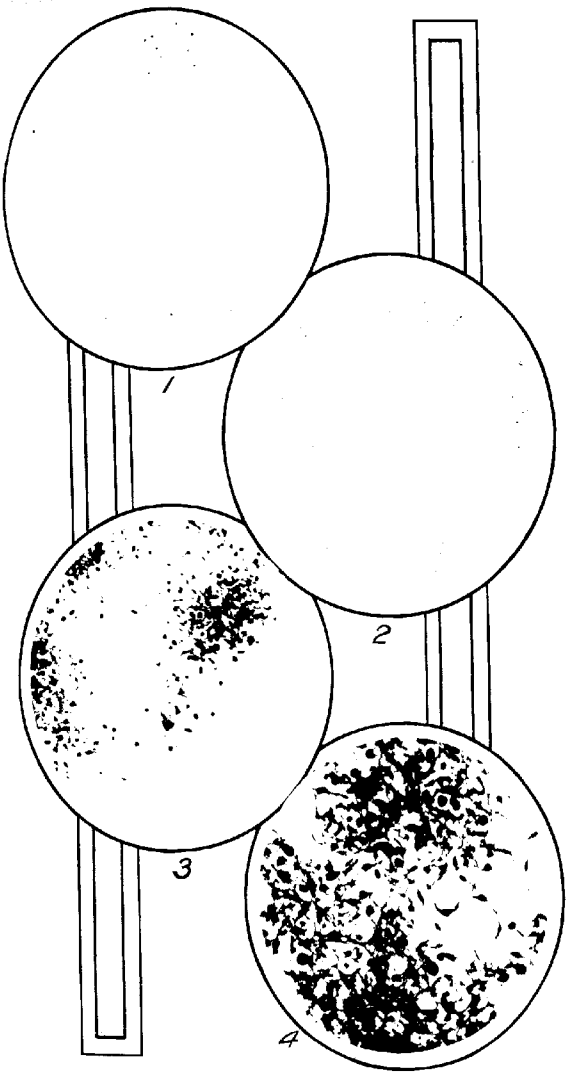
PLATE XV

Fig. 1.—Germinating gonidia of the potato-scab organism, agar hanging-block, 3 hours' incubation at 35° C. ×375.

Fig. 2.—Germinating gonidia of the potato-scab organism, agar hanging-block, 5 hours' incubation at 35° C. ×375.

Fig. 3.—Involution forms of the potato-scab organism on synthetic agar from a 1-month-old culture, stained with carbol fuchsin. ×425.

Fig. 4.—Involution forms of the potato-scab organism on synthetic agar from a 1-month-old culture, stained with carbol fuchsin. ×750.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SEEDLING DISEASES OF SUGAR BEETS AND THEIR RELATION TO ROOT-ROT AND CROWN-ROT[1]

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INTRODUCTION

The diseases of the sugar beet (*Beta vulgaris L.*) to be discussed in this paper are damping-off and a more common but less familiar seedling trouble which will be designated as "root sickness," together with the associated rots of the growing or of the mature root.

Damping-off is a disease which typically manifests itself by a characteristic browning or blackening of that portion of the root or hypocotyl near the surface of the ground. Plants may either be killed by the progress of the disease or checked in growth for a longer or shorter time, according to the severity of the attack and the environmental conditions. The trouble is found in incipient form wherever the host is cultivated and not infrequently becomes epidemic, completely destroying the stand.

Root sickness is similar in some respects to damping-off, but the attack in this case is confined to the root system, seldom appearing aboveground. For this reason it has heretofore escaped general recognition as a distinct disease and has received no treatment in American literature. Diseased plants assume a slightly flabby appearance and are perhaps a trifle lighter green than normal ones. In severe cases the entire field may be wiped out, but more frequently enough plants survive to produce at least a partial stand. The beets make practically no growth during the continuance of the attack, which is usually for two or three weeks. By carefully removing the sick plants from the soil so as to avoid breaking the roots it may be seen that the side branches and taproots are blackened, shriveled, and more or less completely killed. Healthy new shoots are sent out here and there from the upper portion in an attempt at recovery. If the plant survives, one of these eventually replaces the taproot. However, such beets are not only delayed two or three weeks in growth but they are likely to be short and branching. The stand is always more or less imperfect in fields that have recovered from the disease. This type of trouble is almost universal, and in some of the heavier soils of the more northern areas it frequently becomes a limiting factor in sugar-beet production. Even in the more favored sections it is annually the cause of

[1] The major portion of the work reported in this paper was carried out at Madison, Wis., in cooperation with the Wisconsin Agricultural Experiment Station, which kindly supplied space and care for the field plots and provided all necessary laboratory and greenhouse facilities.

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serious losses. Many of the failures attributed to faulty germination are, in fact, the results of serious outbreaks of this disease, and in practically all the cases investigated where seedlings were "not growing well" the trouble has been found to be root sickness.

These or similar diseases have been known for a long time in Europe, where various and widely different theories have been advanced regarding their cause or causes. Refractory soil, cold ground, wet weather, poor cultivation, excessive rain, fungus infection, and the like have all had their advocates, who have based their opinions in many instances upon insufficient data.

Hesse (24) [1] reported the presence of *Pythium debaryanum* in diseased beet seedlings in 1874, but he seems to have done no experimental work with this host. Hellriegel (23), one of the first investigators who made a careful experimental study of this subject, showed that damping-off in his pot experiments appeared to be due to a parasite, and traced the source of infection to the seed. He did not, however, assign a specific organism as the cause. Eidam (13) produced artificial infection of beet seedlings with cultures of *Rhizoctonia betae* Kühn. Krüger (25, 26) found *Phoma betae* Fr. to be an active seedling parasite of the sugar beet and expressed the opinion that several different fungi are capable of producing diseases in seedlings of this plant so similar in appearance that they have been classed together under the name "Wurzelbrand." Several fungi, as well as bacteria, have since been added to this list of parasites, while other writers have denied the parasitic origin of the disease.

So much contradiction and uncertainty exists in the literature of the last 20 years regarding the nature and cause of Wurzelbrand of beets in Europe that Peters (31, 32, 33) and his associates (5, 6) found it necessary to go over the entire subject and submit to rigid experimental proofs the more worthy of the hypotheses that have been put forth.

From what appears to be a careful and trustworthy piece of work, Peters (33) concludes that under the conditions of his experiments *Pythium debaryanum* Hesse, *Phoma betae* Fr., and *Aphanomyces laevis* De By. are capable of producing damping-off of the sugar beet. He was unable to secure pure cultures of *Rhizoctonia violacea* Tul., but he infected soil with fragments of beets showing typical Rhizoctonia decay and failed to produce damping-off. The evidence of the parasitism of bacteria on this host seemed to him insufficient to justify serious consideration.

In America the only reported work on seedling diseases of beets, except the author's preliminary note (12), is by Duggar (9, 11), who conducted successful infection experiments with a species of Rhizoctonia that he secured from decayed beets and later designated as *Corticium vagum* B. and C., var. *solani* Burt. (10, p. 444–452). His experiments were carried out in sterilized soil and controlled by check plants

[1] Reference is made by number to "Literature cited," p. 165–168.

which remained healthy, but he makes no mention of treating the seed to insure the elimination of *Phoma betae*, which he regarded as absent (10, p. 344).

THE SECURING OF CULTURES

The first steps in the present work were naturally the securing of cultures, which were obtained in various ways. Beet seed was placed in sterilized filter papers in sterile moist chambers, and transfers were made from the colonies of fungi which developed during the progress of germination. This method is uncertain and yields a large number of harmless saprophytes. Seed was sown in soil which had been sterilized in an autoclave at 12 pounds' pressure for from four to six hours on two successive days. These were watered with sterile water and protected from outside sources of infection. Whenever damping-off appeared, the diseased seedlings were removed and treated for one minute with more or less shaking in a solution (1:1,000) of bichlorid of mercury in water or in similar bichlorid solutions containing either 1 gm. of ammonium chlorid or ½ c. c. of concentrated hydrochloric acid per liter. They were then rinsed in sterilized water and dropped upon suitable nutrient-agar medium in Petri dishes (Pl. XVI and XVII). The agar most commonly used is sufficiently acid to materially check the development of bacteria and is at the same time a very satisfactory medium for the cultivation of most fungi. It has the following composition:

Dextrose	100	Dipotassium hydrogen phosphate	2.5
Peptone	5	Calcium chlorid	.1
Ammonium nitrate	10	Water	1,000
Potassium nitrate	5	Agar	20
Magnesium sulphate	2.5		

As soon as growth appeared from the seedlings, isolation transfers were made. Cultures obtained by these two methods were regarded as originating from the seed.

Cultures were made from the soil indirectly by means of seedlings in the following manner: Beet seed, treated by a method to be discussed later, so as to insure the absence of parasitic fungi, was sown in unsterilized soil in pots that were thoroughly sterilized before using. When damping-off occurred, isolations were made in the manner already described. Cultures were secured from decayed beets by cutting out with a sterile knife small portions of material on the border line between healthy and diseased tissue. These blocks were placed upon a suitable medium, and isolations were made from the developing colonies. Another method employed was to sow treated beet seed in sterilized soil, subsequently infected with fragments of decaying beets. Isolations were then made from the seedlings when disease developed. Large numbers of isolations were made from sugar-beet seedlings grown in commercial fields, and a considerable number of cultures were courteously contributed by various workers from time to time.

SEED TREATMENT

Sugar-beet seed is quite universally infected with parasitic fungi. It was therefore necessary to devise some method of freeing the seed from infection before inoculation experiments could be successfully conducted. Among the substances tried were hydrogen peroxid, hydrochloric acid, sulphuric acid, formalin solution, formaldehyde vapor, and hot water. Peroxid solution (6 per cent) was used for varying periods up to one hour. The seed was then sown in sterilized soil and watered with distilled water. The pots were protected from infection, but damping-off was in no degree checked, and *Phoma betae* was invariably isolated from the diseased seedlings (Pl. XVII).

Hydrochloric acid was employed in various concentrations up to a specific gravity of 20° B. for 15 minutes. The seed was then rinsed in sterile water, followed by lime water, which in turn was followed by sterile water. This treatment was without effect upon the vitality either of the seed or of the fungi.

Sulphuric acid was used in various strengths up to full concentration for one hour. The treated seed germinated strongly and from 24 to 48 hours earlier than the untreated control, but there was no decrease in the amount of damping-off.

Formalin solution was employed up to concentrations of 2 per cent of formaldehyde for various intervals up to one hour. This seriously injured the viability of the seed, but afforded no check to the disease. The results with formaldehyde vapor are inconclusive, since they lack uniformity.

The method finally settled upon for experimental work was one employed by Peters (7, p. 273–274), which consists of heating the seed in water at 60° C. for 10 minutes, promptly drying superficially upon filter papers, so as to prevent germination, and after an interval of 24 hours heating a second time for 10 minutes at 60° C. The seed treated in this way and sown in sterilized soil, watered with sterile water, and protected from outside infection remained practically free from disease. Not more than one seedling in three or four hundred was infected with *Phoma betae*. The percentage of germination is unquestionably lowered by this treatment, but it is the only method tried by which inoculation experiments could be controlled. It does not appear to be a method which could be applied on a commercial scale.

METHODS OF INOCULATION

Inoculation experiments were carried out in pots either in the laboratory or usually in the greenhouse, using seed treated in the manner just described, and with soil sterilized by heating three or four hours in the autoclave under a pressure of from 12 to 15 pounds on two, or usually three, consecutive days. Inoculations were made in the soil or upon the seed at the time of sowing, except when otherwise stated. Various

methods of inoculation were employed, but the results were uniformly the same. Either suspensions of spores or mycelial growth on various culture media, such as agar, corn meal, beet petioles, and sterilized beet blocks, were employed. The last method—that is, with beet blocks—was perhaps the most satisfactory and convenient. The corn-meal cultures appeared to exercise an unfavorable physiological action, possibly because of the bacterial growth which they fostered; so this method was discarded.

Inoculation experiments were invariably controlled by a considerable number of uninoculated pots. In a few instances disease occurred in the controls. In such cases the causal organism was determined, but the entire series was abandoned as an inoculation experiment, even though the presence of an intruder could be explained readily through the agency of insects and earthworms. It was the invariable custom to recover the fungus from the damping-off seedlings by the method already described (Pl. XVI and XVII) and to reinoculate and recover through from four to six generations of seedlings.

As reported in a former note (12), four fungi have been found to stand in causal relation to seedling-beet troubles. These are *Phoma betae* (Oud.) Fr., a species of Rhizoctonia, regarded as identical with the form described as *Corticium vagum* B. and C., var. *solani* Burt., *Pythium debaryanum* Hesse, and a fungus originally reported as *Aphanomyces laevis* De By., but which has since been found to be new.

PHOMA BETAE

TAXONOMY

Frank (16, 17, 18, 20) established the relation of *Phoma betae* to heart-rot of the sugar beet in 1892. The following year Krüger (25, 26), working in the same field, demonstrated its causal relation to damping-off. He found the fungus fruiting abundantly on all parts of diseased beets and held it to be identical with the fungus which had previously been observed on various portions of the cultivated varieties of *Beta vulgaris* L.

Oudemans (29, p. 181) had observed what appeared to be the same fungus fruiting upon leaf spots of old beets and applied the name "*Phyllosticta betae.*" Prillieux (35, p. 19) observed the fungus on leaf blades and decaying heart leaves, as well as upon typical spots on the leaf, and applied the name "*Phyllosticta tabifica.*" Saccardo recognizes the names "*Phyllosticta betae* Oud." and "*Phoma betae* Rostr." The latter name is given on the authority of the following paragraph from the pen of E. Rostrup (41, p. 323):

> Eine zweite, an Runkelrüben auftretende *Phoma* habe ich zuerst in meinem Jahresbericht über Krankheiten der Kulturgewächse im Jahre 1888 (Tidsskrift for Landökonomi. R. 5, Bd. 8, S. 746) [40] unter dem Namen *Phoma sphaerosperma* beschrieben. Weil sich aber herausstellte, dass dieser Name schon im Jahre 1885 einer ganz anderen Art gegeben war, nannte ich später den Pilz *Phoma Betae.*

No reference to the publication in which this change of name was announced is given, and efforts to find it have failed. Moreover, Lind (28, p. 415), who had full access to Rostrup's specimens and publications, lists *Phoma betae* Rostr. under *Phoma betae* Fr. as a synonym. Frank suggested the name "*Phoma betae*" in the article previously cited (16), published in 1892, which contains a description of the fungus, accompanied by figures and a somewhat extended discussion of the root-rot produced by it on the sugar beet. In view of the established identity between Phoma and Phyllosticta on sugar beets it would appear that Oudemans' description in 1877 (29) has first claim to priority.

For these reasons it seems proper and convenient to retain the name which has persisted in most general use in literature, but with a correction to insure proper acknowledgment to Oudemans. The name "*Phoma betae* (Oud.) Fr." is therefore used in this paper. Pool and McKay, who agree in the justice of this usage, also employ it in a current paper (34).

Neither Frank (17) nor Krüger (25, 26) were able to find evidences of a perfect stage of this fungus in their cultural studies. Peters (31, 32, 33) also failed to find sexual fruits, and the same is true of the work of the writer. It should be pointed out, however, that Rostrup (40, p. 746) believed *Sporodesmium putrefaciens* Fuck. to be a perfect stage of *Phoma betae*, and Prillieux and Delacroix (37) regarded *Phoma betae* as the pycnidial form of *Sphaerella tabifica* Delacr.

IDENTITY OF PHOMA AND PHYLLOSTICTA ON THE SUGAR BEET

Hedgcock (22) has presented evidence by cross-inoculation of the identity of Phyllosticta and Phoma on the sugar beet. He grew beets from seed treated with concentrated sulphuric acid for 30 minutes, followed by an alkali, and successfully produced Phyllosticta spots by spraying upon the leaves spores of a Phoma culture isolated from decaying beets. Beets whose leaves were covered with Phyllosticta were placed in a dry cellar and held under observation for two months, during which time the characteristic black rot of Phoma passed from the leaf petioles to the crown of the beet.

While Hedgcock's conclusions are undoubtedly correct, the seed treatment he employed does not destroy the viability of Phoma, and it will be shown later that beets whose foliage was free from visible evidence of Phyllosticta decayed from Phoma when they were placed in a relatively dry environment. The case may be strengthened, therefore, by the presentation of the additional evidence now available. Cultures from Phyllosticta spots, as well as from decayed beets and from damped-off seedlings, have been found equally capable of producing damping-off of sugar beets, and a somewhat extended study of the morphology of the fungus from the three sources mentioned has revealed no differences

between them. The Phyllosticta cultures employed were supplied by Miss Venus W. Pool, of the Rocky Ford (Colo.) field station. Pool and McKay (34), who worked with Phoma cultures isolated by the writer from decayed sugar beets, found them capable of producing the characteristic leaf spots as readily as cultures isolated from the Phyllosticta pycnidia. The fungus, however, is not an aggressive leaf parasite, but does its greatest injury on the root.

SOURCES OF INFECTION

The source of original infection appears to be the seed. It has been generally recognized in Europe for years that seed infection with *Phoma betae* is universal. As American growers are using European seed almost exclusively, it follows that the disease is constantly being introduced into the United States on seed. A very large number of samples of both European- and American-grown beet seed have been examined for the presence of this and other pathogenic forms. With the exception of one single lot of seed, the examination of 100 seed balls by the seedling method has invariably demonstrated the presence of *Phoma betae*. Reexamination of this one lot, which was American-grown, revealed the presence of the fungus in it also when larger samples were tested.

Frank (18, p. 180, 272–293) believed the fungus capable of living over in the soil by means of its spores, but Peters (7, p. 278–286) holds that it can do so only when fragments of beets are present to support mycelial growth. A large number of trials of soil in America made by means of seedlings growing in it from pasteurized seed indicate that Phoma does not remain viable in the soil after the decomposition and disintegration of its host. Field soils containing decaying beet fragments occasionally yield cultures of the fungus in the spring of the first year following beets, but, as a rule, even seriously beet-sick soils fail to give them. Samples have been examined from Virginia, District of Columbia, Michigan, Wisconsin, Kansas, Colorado, Utah, and California.

MORPHOLOGY OF THE FUNGUS

Several hundred cultures of the fungus have been isolated and grown upon media (Pl. XVII). No constant differences in cultural characters of strains from the various sources have been observed. It is readily cultivated upon a great variety of media, although on many of these it develops mycelium only. It fruits abundantly upon string-bean agar (Pl. XVII, fig. 2) and this medium has been used for purposes of identification and for measurements of pycnidia and spores. It is evident that the curves which might be plotted from the following tabulated results of measurements would be irregular and consequently that they would probably be changed by increasing the number of pycnidia and spores

measured (Table I). Of 181 pycnidia measured the smallest was 125μ and the largest 635μ in diameter. The largest number fall between 225 and 325μ.

TABLE I.—*Variation in size of 181 pycnidia of Phoma betae*

Number measured.	Variation in diameter.	Number measured.	Variation in diameter.
	μ		μ
1	125	11	351 to 375
4	126 to 150	5	376 to 400
14	151 to 175	10	401 to 425
7	176 to 200	5	426 to 450
31	201 to 225	1	451 to 475
21	226 to 250	1	476 to 500
10	251 to 275	1	501 to 525
27	276 to 300	1	526 to 550
19	301 to 325	1	551 to 635
11	326 to 350		

The pycnospores showed quite wide variations in size. The shortest of 204 spores measured 3.8 and the longest 9.4μ. Practically all fell between 4.1 and 7μ, as shown in Table I.

The width of the pycnospores varied from 2.6 to 4.3μ. The single exception to this case is a spore which measured 4.9μ in width. Table II will show the distribution of numbers within the limits given.

TABLE II.—*Variation in size of 204 pycnospores of Phoma betae*

LENGTH

Number measured.	Variation.	Number measured.	Variation.
	μ		μ
1	3.8	14	6.1 to 6.2
4	4.1 to 4.2	11	6.3 to 6.4
3	4.3 to 4.4	6	6.5 to 6.6
2	4.5 to 4.6	6	6.7 to 6.8
14	4.7 to 4.8	7	6.9 to 7.0
13	4.9 to 5.0	1	7.1 to 7.2
22	5.1 to 5.2	1	7.3 to 7.4
21	5.3 to 5.4	2	7.5 to 7.6
24	5.5 to 5.6	2	7.7 to 7.8
22	5.7 to 5.8	1	7.9 to 8.0
25	5.9 to 6.0	2	9.3 to 9.4

WIDTH

5	2.6 to 2.7	22	3.6 to 3.7
15	2.8 to 2.9	17	3.8 to 3.9
43	3.0 to 3.1	7	4.0 to 4.1
49	3.2 to 3.3	3	4.2 to 4.3
42	3.4 to 3.5	1	4.9

VITALITY IN CULTURE

Phoma betae exhibits long vitality in culture, as is shown by the following tests.

On July 2, 1913, old cultures which had been apparently air-dry for months were opened under sterile conditions, and into each a portion of string-bean agar was introduced. The tubes were then placed to harden in such a position as to leave the old culture partly submerged. Table III gives the age of the cultures and the results after six days' incubation.

TABLE III.—*Vitality of Phoma betae in culture*

Phoma strain.	Number.	Age of culture.	Results.	Phoma strain.	Number.	Age of culture.	Results.
		Days.				*Days.*	
Strain 3...	39	413	Good growth and pycnidia.	Strain M..	1,304	315	Good growth and pycnidia.
Strain A...	423	372	Do.	Strain 3...	64	413	No growth.
Strain J...	564	358	Do.	Strain 3...	40	413	Do.
Strain J...	996	315	Do.	Strain 3..	281	404	Do.
Strain E..	1,296	315	Do.	Strain 3..	386	404	Do.
Strain J..	1,301	315	Do.				

INOCULATION EXPERIMENTS ON SEEDLINGS

The strains used in inoculation experiments are 24 in number, obtained from the following sources:

Direct isolation from sugar-beet seed by the moist-chamber method, 1.

Indirect isolation from sugar-beet seed through damping-off seedlings in sterilized soil, 2.

Direct isolation from Phyllosticta leaf-spot, 2.

Isolations from various sources such as beet leaves, beet seed, and beet-sick soil at Rocky Ford, Colo., 13.[1]

Isolations from beets having heart-rot, from Colorado, 1; Wisconsin, 2; South Dakota, 1.

Isolations from seedlings grown in sterilized soil, inoculated with decayed beets, 2.

The inoculation experiments upon seedlings were carried out in the manner already described, invariably yielding positive results. There appeared to be no decrease in virulence from carrying the fungus in culture 14 months.

The first development of damping-off in the pots occurred usually about the third day after the seedlings broke the ground and continued till about the time they developed their third or fourth pair of leaves (Pl. XVIII, fig. 2). The method followed was to sow 100 seed balls per

[1] These cultures were kindly supplied by Miss Venus W. Pool, of the Bureau of Plant Industry, from the Rocky Ford (Colo.) field station.

pot and to examine daily for damping-off. Whenever disease occurred, the plants affected were removed and a record made of their number. The period of greatest susceptibility seemed to be passed by the time the third set of leaves appeared. After a sufficient period the remaining plants were harvested and examined for signs of infection on the roots.

The following record (Table IV) of a typical series illustrates the method and gives a good idea of the average results.

TABLE IV.—*Results of inoculation experiment with Phoma betae*

[Series of June 27, 1912]

Phoma strain.	*Appeared above-ground on—*	Number of seedlings diseased on July—									Number harvested on July 29	
		3	4	5	6	7	10	12	15	17	Diseased.	Healthy.
Strain C	July 1			3	5	6	18	19	5	0	4	18
Strain E	do		7	10	7	8	9	7	3	2	5	3
Strain B	do		2	3	6	3	12	13	4	4	7	30
Strain A	July 2		1	2	8	12	27	17	6	2	7	13
Strain D	July 1	2	24	20	13	8	16	2	0	0	1	0
Strain F	do				5	6	9	9	10	1	5	14
Strain H	do		7	44	12	0	11	0	0	2		
Strain G	do				8	0	21	18	14	0	52	12
Do	do		5	11	22	45	5	0	0	0	5	7
Control	July 2											111
Do	do											148

The plants described as diseased were typical root-sick beets. There was nothing about their appearance aboveground in the pots to indicate the presence of the fungus, but in the field such seedlings may often be detected from the fact that they appear to be suffering from lack of moisture. This type of disease is almost universal under field conditions and is far more common than damping-off. Histological studies upon such material, to be published in a subsequent paper, revealed the significant fact that it often carries the pycnidia and sometimes the vegetative mycelium of the fungus.

The number of seedlings developing in control pots was, as a rule, larger than the total number developing in inoculated ones. This is to be explained by the fact that some of the seedlings were attacked before they broke the ground and in consequence were unable to push through the soil. A careful examination of the surface soil in inoculated pots now and then revealed such seedlings in a state of more or less complete decay.

PHOMA FIELD-ROT AND STORAGE-ROT FOLLOWING SEEDLING INFECTION

When diseased plants were allowed to remain in the pots and were held under as favorable conditions as possible, a large proportion of them eventually survived and sent out new roots. This condition is

often observed in commercial fields, and it is probably safe to say that, under advantageous conditions of climate and cultivation, 75 per cent of the beets attacked by *Phoma betae* are not killed, but throw off the parasite sufficiently to make a good growth while continuing to carry the fungus in a dormant condition upon the crown. When the vitality of the host is sufficiently reduced, the parasite may become active again and develop either in the root, where it causes a black rot, or on the aerial portions of the plant, where it causes a spot disease, which, while it does not injure the host to an appreciable extent, enables the fungus to infect the seed growing on mother plants. The rot is, of course, more serious to the plants attacked. Cases of this so-called heart-rot in the field have been very destructive at times in certain sections of Europe and are not infrequent in America. Important instances of its occurrence in Michigan, Wisconsin, and Colorado, and a less serious case in New York, have come to the writer's attention. Material from the three States first mentioned was available for study. The fungus was easily secured in pure culture from the beets that were partially decayed by the black rot typical of the disease (Pl. XVIII, fig. 1). Many roots presented unmistakable evidence of having been diseased in the seedling stage. In some cases the original taproot had been destroyed, and only a knob of tissue just below the crown had survived.

Additional evidence that the fungus is capable of living upon its host for a long time in an inconspicuous and apparently harmless form until those conditions appear which are favorable to its development is readily found upon examination of beets in storage or those which have been kept over for seed purposes. Such examination in the spring has never failed to reveal the presence of the fungus. Sometimes the entire root is destroyed, but more frequently beets that are apparently healthy may be found to show black spots in certain sections in or near their vascular tissue, especially in the top of the crown. These areas usually develop longitudinally and are frequently confined to the vicinity of a single vascular bundle (Pl. XIX). When the conditions of storage have been unfavorable, the rot may assume an epidemic form and lead to the total destruction of roots which were apparently healthy and sound when placed in storage.

INOCULATION EXPERIMENTS ON GROWING BEETS

Evidence that the infection takes place only in the seedling stage or through the leaves in old age is found in the results of inoculation experiments made with cultures of *Phoma betae*. In August, 1912, 100 apparently healthy beets growing in the field at Madison, Wis., were inoculated. A portion of the infections were made directly upon the uninjured crown with vegetative cultures upon beet blocks. Other inoculations were made through wounds near the surface of the soil and

still others through wounds on the petiole or in the heart of the crown. In no case was there any evidence of rot in the field or at the time of harvest. It was at first believed that climatic conditions were responsible for the failure. However, the beets were placed in storage. The following spring they were examined for evidences of *Phoma betae* by culture methods. None of the beets had been destroyed; indeed, most of them appeared to be perfectly sound until they were opened, when the black lines previously mentioned appeared in the vascular regions. Cultures were obtained from each of the infected beets, except two. These results were at first interpreted as an indication that the inoculations made in August had produced infection which had persisted on the surface and penetrated the roots in storage, but subsequent developments throw doubt upon this conclusion and indicate that the infection occurred much earlier. About half a ton of beets grown as controls had been placed in storage beside the infected material, but in a lower rack. Evidences of rot were observed in only half a dozen of these beets, and they yielded cultures of *Phoma betae*. It was noted at the time of examination that the control beets, which had been kept nearer the surface of the soil, were all firmer than the inoculated material; but unfortunately no significance was attached to this fact at the time, and, with the exception of 12 of the control beets, the entire lot of material was sent to the feeding sheds.

A few weeks later a large quantity of mother beets grown in the vicinity of the experimental field from western seed was found to be seriously injured by the Phoma rot, practically every beet showing more or less evidence of it. These beets had been bored for analysis in the fall, so that any evidence of infection at that time would have been apparent. The decay did not originate from infection in the wound made by boring, as may be seen readily by reference to the illustration (Pl. XIX). In the large majority of cases the rot evidently started from the crown, although there were cases of pockets of decay on the sides or even at the tip of the beet. In some instances dark streaks could be traced from the crown to decayed areas in the lower portion. This led to the belief that the decay in beets inoculated with *Phoma betae* was due to infection occurring in the seedling stage and that its development was fostered by the less favorable, dryer conditions of storage to which they were submitted. Additional evidence for this belief is to be found in the results of the inoculation experiments with Rhizoctonia (p. 153), since the Phoma rot appeared upon this material also. Moreover, of the few control beets saved from the feeding sheds all but three developed Phoma decay during the late spring and early summer, and cultures of *Phoma betae* were secured from darkened bundles in the crowns of these three. It might be urged that the infection reached the crowns of these beets by way of the petioles from Phyllosticta upon the leaves. This seems a very probable means of infection, but appears unlikely in the instances above cited,

since Phyllosticta spots were at no time observed upon the foliage of the beets in question, which were grown in isolation and were under daily observation. The following season (1913) inoculations were made on beets grown outdoors at Madison, Wis., in pots filled with soil from Garden City, Kans., from Rocky Ford, Colo., and from Madison, Wis. Other beets grown in the usual way in the field at Madison, at Garden City, and at Rocky Ford were inoculated. In all cases this was done by placing a large fragment of actively growing culture on sterilized beet blocks upon the crown of the beet near the heart and a second portion in direct contact with an abrasion on the crown just beneath the surface of the soil. Through the courtesy of colleagues at Garden City and Rocky Ford it was possible to have the beets watched at these points as carefully as were those at Madison. At no time during the season did evidence of infection appear on any of the beets under observation.

Two lots of beets were grown in 1913 from pasteurized seed. One lot was isolated from other beets so as to be protected from infection. These beets were stored in the fall in a warm basement, which offered very favorable conditions for the development of Phoma storage-rot, as shown by previous experience. They were examined from time to time during the winter, spring, and summer for evidences of *Phoma betae*, with negative results.

The other lot of seed was sown in proximity to breeding stock and mother plants known to be infected with *Phoma betae*, and stored in a cellar with infected material. These beets developed Phoma rot during the winter. Phyllosticta appeared on seed plants grown from some of these roots during the summer of 1914, and the seed produced was found to carry the pycnidia and spores of Phoma.

PERPETUATION OF THE FUNGUS

The results of the various experiments coupled with field observations lead to the opinion that *Phoma betae* is capable of infecting *Beta vulgaris* L. only during periods of especial susceptibility, such as the early seedling stage, or, in the case of the leaf, during physiological old age. It may also be that the root is susceptible to infection during periods of low vitality induced by unfavorable environment, but experimental proof is lacking. It does appear, however, that this fungus is entirely capable of living for a long time in a hidden condition upon the crown of the beet, ready to take advantage of any diminished vitality in its host. After infection has once occurred, the parasite may remain present in a viable though inconspicuous condition, although the host appears to have completely overcome the disease. The Phoma field-rot in the summer and fall of 1913, in Wisconsin and Colorado, respectively, developing in the first instance under conditions of excessive moisture and in the second under severe drought, may be explained in this way. The crown

infections originating from the seed readily explain the source of the spores producing the outbreaks of Phyllosticta, which appear to be much more common in regions of low humidity, where, as Pool and McKay (34) have shown, viable spores of this fungus are common in the air. In this connection, attention should be especially called to observations during the seasons of 1912 and 1913 in Colorado, Idaho, and Wisconsin, since they indicate a direct connection between the occurrence of Phyllosticta on the leaves and seed stalks of mother beets and the presence of Phoma on the seed they produce. Phyllosticta was quite prevalent in the Idaho-Colorado region, but escaped detection by the same observer in Wisconsin. Tests upon seed produced in these regions during both seasons have shown that the more western seed was quite generally infected to an extent comparable to that from European sources, while that grown in Wisconsin showed only a very slight infection.

Pool and McKay, who kindly continued the observations at Madison during the absence of the author in the season of 1914, found the Phyllosticta form on the leaves and stalks of seed beets and also on first-year beets. In the absence of fruits the causal fungus was identified by cultural methods. In other instances pycnidia were produced. The spots were, as a rule, less clearly defined and less numerous than those in the Idaho-Colorado region, which are similar to those seen in Europe in 1914, where they were common on both field and mother beets. Since the leaf form of the disease occurred in Wisconsin in 1914, it is probably safe to assume that it existed there on seed beets, undetected, in the two preceding years. However, if the absence of Phyllosticta infection in Wisconsin in 1912 and 1913 be assumed, the presence of the small amount of *Phoma betae* on the seed produced there may still be accounted for in at least three different ways. The seed balls may have been infected at any time between blossom and maturity by air-borne spores developing in the crown. The fungus may have spread from the crown of the mother plants on the surface of the seed stalks to the racemes and infected the fruit, or it may have passed through the vascular system of the stem to the seed.

CONTROL OF THE FUNGUS

It is apparent that *Phoma betae* is one of the most serious obstacles which the growers of beet seed in America have to face. Its ravages on mother beets during the winter may be largely overcome by favorable conditions of storage, but in case the mother beet escapes destruction the infection remains to proceed to the leaves and seed stalks and to infect the seed. Since the fungus sometimes inhabits the vascular region of the root, it may possibly progress through the stem, as well as on the surface. In any event, it is an undoubted fact that it finds its way into the young seed ball, where it starts with the seed upon another 2-year cycle.

From what has been said regarding sources of infection, it appears that ordinary attention to rotation should eliminate danger from soil infection, but that all seed at present available is heavily infected. The only hope of control therefore lies in one or more of three alternatives: (1) The natural resistance of the beet to the attacks of the fungus, (2) seed treatments, (3) the production of disease-free seed.

From the facts that the period of infection with *Phoma betae* is normally confined to a relatively short period in the seedling stage and that infected plants frequently throw off the attack, proper attention to cultural conditions would seem to offer hopeful prospect of control. European experience has demonstrated the value of such methods. Indeed, some prominent agriculturists have denied the pathogenicity of *Phoma betae* because of their success in preventing damping-off and root sickness by proper cultivation and fertilization. From this they have argued that the cause lies in unfavorable cultural conditions rather than the presence of parasites. The truth undoubtedly is that unfavorable environment is a predisposing cause which so weakens the beet that it is unable to compete successfully with the fungus. When planted in good soil, which has been well prepared and suitably fertilized, the seed germinates promptly and the young plants pass rapidly through the period of danger. Early cultivation at this stage to insure proper aeration of the roots is beneficial. The studies upon control by means of cultural methods and fertilizers demonstrate the value of properly prepared soil and thorough cultivation promptly after the seedlings come through the ground. There is no doubt that frequently an infected stand may be saved in this way. In Europe the use of phosphoric acid and potash has given good results. The question of soil reaction has also been found very important. Applications of lime on certain types of acid soil result in almost complete control. This may also prove to be the case in America, but the point can be determined only by local experiment.

As has already been said, the only method of seed treatment which has given satisfactory results in pot experiments is seed pasteurization. Experimental work to test the efficiency of this method in field practice has been attempted. It is not believed that a method of treatment as difficult to carry out as pasteurization will prove useful except in the hands of experimentalists and for experimental purposes. It is apparent that seed must be treated in small lots and with extreme care in order to secure the desired result. If temperatures much above 60° C. are employed, the injury to the seed becomes serious. Temperatures below 60° are ineffective. The substitution of one treatment for two is likewise unsuccessful. Attempts to apply the treatment to samples of seed of even 5 pounds have not been altogether satisfactory, but appear to hold the fungus in check.

One experiment in seed treatment carried out under field conditions where epidemic development of root sickness has annually occurred for

several years will illustrate the point. A half-acre field was prepared. Five pounds of pasteurized seed were sown on one quarter-acre and five pounds of untreated seed on the other. As soon as the seedlings were well out of the ground, examination was made for root sickness and damping-off. No signs of damping-off were seen, but considerable root sickness was in evidence on both the treated and untreated sections. Cultures were started in the field by treating the seedlings for one minute in a triturate of citric acid and bichlorid of mercury in water of such strength as to give a 1 to 1,000 solution of sublimate. They were then transferred to test tubes of sterilized water and were brought to the laboratory, where they were plated. A period of 36 hours elapsed between the treatment with the bichlorid of mercury and the plating. From the untreated seed 95 seedlings yielded the following results: Phoma, 29; Fusarium, 19; Macrosporium, 2; Mucor, 6; miscellaneous, 18; no growth, 21. From the treated seed 69 seedlings yielded: Fusarium, 23; an unidentified ascomycete, 11; Macrosporium, 2; Mucor, 2; Penicillium, 1; miscellaneous, 8; no growth, 22. *Phoma betae* was not found in the treated lot. The long interval before plating undoubtedly accounts for the large amount of Fusarium and perhaps also for the failure to secure growth in many cases.

The average results secured from prompt plating may be seen from the following series, which was from the same locality but was made two weeks earlier. Many of these were plated immediately. Most of the Fusarium resulted from seedlings which were carried in the water blanks for half a day or more. Two hundred seedlings yielded cultures as follows: Phoma, 149; Fusarium, 29; Pythium, 3; miscellaneous, 11; no growth, 8. It therefore appears that, while seed pasteurization may be employed successfully to rid seed of *Phoma betae* for experimental purposes, it is not applicable on a commercial scale. Moreover, such treatment does not guarantee freedom from physiological root sickness associated with saprophytic fungi, since the vitality of the seedlings seems to be lowered by pasteurization.

A realization of these facts suggests the necessity of clean stock for breeding purposes. It would seem that if the growers of elite strains could rid their stock of this parasite it would thereafter remain clean, provided a reasonable rotation were observed and the seed fields were sufficiently isolated to escape reinfection with Phyllosticta. Danger from this source would diminish with the increase in the supply of clean seed. The experimental work already reported has shown the possibility of eliminating the fungus from plants grown in isolation from pasteurized seed. These plants would produce clean seed which could again be sown in isolation from infected stock and made the basis for a seed supply for an entire community, from which the fungus would be eliminated. This community could be employed as a breeding center where the entire seed supply of a factory could be grown, and one of the most

serious fungous pests of the sugar beet eliminated from its territory. Since sugar companies have absolute control over the sources of seed supply of their growers, it is quite possible for a company producing even a portion of its own seed to maintain an area of quarantine within any portion of its territory where it does not compete with other companies for acreage, provided table beets and mangel-wurzels are not allowed to bring in the infection.

RHIZOCTONIA

The genus Rhizoctonia has been used to include a group of sterile fungi, more or less closely related morphologically. Much confusion exists regarding the identity of the various forms, and there is likewise great diversity of opinion as to the pathogenic properties of the members of the group. To make clear, especially to foreign investigators, the identity of the fungus under consideration in this paper, a brief discussion of the literature seems essential. The name "Rhizoctonia" was first applied by De Candolle (8) in 1815 to a fungus on alfalfa. He eventually distinguished three species, *R. crocorum*, *R. medicaginis*, and *R. mali*. During the following 35 years various workers described a series of diseases caused by similar fungi, which were referred to this or other genera. In 1851 Tulasne (43, p. 188) united the known forms of Rhizoctonia into one species under the name "*Rhizoctonia violacea*." This classification has been followed by many workers.

In 1858 Kühn (27, p. 222–249) published an account of three species, *R. solani*, *R. medicaginis*, and *R. crocorum*. He distinguished between the two forms first mentioned by the difference in appearance of the sclerotia, those of *R. solani* being smooth, and those of *R. medicaginis*, woolly. He mentions *R. medicaginis* as being parasitic on the beet and carrot, as well as alfalfa, and states that the fungus produces a reddish brown or purplish color in the cells of the beet. This is the first mention of Rhizoctonia on the beet, and it is likewise the first mention of the fungus in Germany. Saccardo has included Kühn's species under *R. violacea*, while Güssow (21), who described a disease on potatoes and alfalfa in England due to Rhizoctonia, considers *R. solani* Kühn to be identical with *R. violacea* Tul. Eriksson (14) in 1903 published the results of inoculation work on various hosts, including the sugar beet. He designated his fungus as *R. violacea*, but later reclassified it as *Hypochnus violaceae* (Tul.) Eriks. (15, p. 421–430). He believed there were biological forms of it, since the form on carrot attacked the beet with more virulence in the second generation than in the first in which it was carried on that host.

Atkinson (1) in 1892 described a damping-off of cotton due to a sterile fungus later classified as Rhizoctonia. Balls (2, 3) has found the same disease in Egypt, and Shaw (42) has more recently reported it from India. In the meantime Duggar (10, p. 344) described the same fungus as a damping-off parasite of the sugar beet, and Pammel (30)

reported a root-rot of beets in Iowa that he believed to be due to *Rhizoctonia betae* Kühn. The fungus, however, appears to be indistinguishable from the one which Atkinson and Duggar, respectively, had reported as a damping-off agent on cotton and sugar-beet seedlings and from forms of Rhizoctonia upon a variety of hosts throughout the United States, acting either as damping-off agents or as the causes of other forms of plant diseases. Rolfs (38, 39), working with the fungus on the potato in Colorado, found a fruiting stage which he designated as *Corticium vagum* B. and C., var. *solani* Burt. He was unable to produce the Corticium in culture, but the growth from spores yielded typical Rhizoctonia mycelium, and infections on living plants with Rhizoctonia gave rise to Corticium. Shaw observed the fruiting stage on the groundnut and later succeeded in producing it by artificial infection on that host. European workers have referred what appears to be the same basidial form to *Hypochnus solani*.

Shaw (42) found marked differences in the character of sclerotia produced by his strains. One which formed small black sclerotia more or less differentiated into cortex and medulla he designated as *R. solani* Kühn. The structure of the sclerotia of the other Rhizoctonia which he designated only as *Corticium vagum* B. and C. appears to correspond closely with that of those obtained in America in cultures and less frequently on the host. He believed this Corticium to be identical with the form common on potatoes in America, but was unable to see justification for referring it to *R. solani* Kühn. Furthermore, he believed *R. violacea* Tul. to be a compound species, possibly including *R. solani* Kühn and the Corticium, since Prillieux (36, t. 2, p. 144) described *R. violacea* Tul. as possessing two distinct forms of sclerotia, one of which, according to Shaw, is similar to those of *R. solani* Kühn and the other to *Corticium vagum*.

A form of crown rot on the sugar beet caused by *Rhizoctonia violacea* Tul. is well known in certain sections of Europe, but rot from *Rhizoctonia solani* is unknown there. This fact has led many students to question the identity of the fungus causing the rot of the beet in America. The organism here considered is distinctly different from the *Rhizoctonia violacea* Tul. type as the writer saw it in Europe on living or preserved material from a variety of hosts, including beets, carrots, potato tubers, alfalfa, and asparagus, but it appears to be identical with the *Rhizoctonia solani* type which forms the sclerotia on the potato in Europe and America. These two fungi when studied on the same host differ in the character of the disease produced, in their appearance on the plants, and in the histological relation of the parasite and host. The two are so distinct in these characters on both the beet and the potato that it seems impossible for one who has seen both types to confuse them. The cultural relations of the two are also distinctly different. *Rhizoctonia solani* is readily cultivated on a variety of media, but all attempts to put

R. violacea into artificial culture have thus far failed, though many different workers have undertaken it. So far as can be determined from the literature, the American fungus also appears to be identical with Shaw's (42) *Corticium vagum* B. and C., and it is indistinguishable in culture from a strain isolated in Ireland by Pethybridge from a single spore of Hypochnus on the potato, and kindly contributed by him under the name "*Hypochnus solani*."

The fungus is characterized by a septate mycelium the branches of which in young cultures are either parallel to or inclined at a more or less acute angle to the direction of growth of the parent branch. There is a constriction where the branch unites with the old hypha and a septum is formed a few microns from the point of origin. The threads are hyaline when young, becoming a yellowish brown with age. In mature cultures the branches are usually arranged very nearly at right angles to the parent thread at the point of origin. In culture and less frequently upon the host it forms sclerotia, which vary greatly in size (Pl. XX, fig. 2). One shown in the illustration of a sugar beet measured a full half-inch (Pl. XXIII). They are usually much smaller, from 1 to 3 mm., and those produced in cultures are likely to be quite irregular in outline. The sclerotia consist of interwoven branches, forming a loose pseudoparenchyma of uniform structure throughout. The sclerotial hyphæ are broken up into short cells each of which may function as a spore when placed under favorable conditions for development. The Corticium stage has not been observed upon the sugar beet, but the fungus appears to be identical with the form on the potato and a variety of other plants upon which the Corticium is common, and the name "*Corticium vagum* B. and C., var. *solani* Burt.," which is the one most generally accepted in America, is being retained for the purposes of this paper.

The beet diseases produced by this fungus in America are unknown in Europe, and this fact has been used as an argument that they can not be correctly attributed here to the fungus which produces the sclerotia on potatoes there. This argument is fully met, however, when we approach a study of the environmental factors upon which the fungus is dependent for the production of disease, since the climatic and soil factors under which it becomes an active parasite in some portions of America are not found in Europe.

INOCULATION EXPERIMENTS ON BEET SEEDLINGS

The 34 cultures used in the inoculation experiments on seedlings were obtained from the following sources:

Sugar-beet seedlings grown in the field at Rocky Ford, Colo., 5; at Madison, Wis., in Rocky Ford soil, 5; at Madison in Garden City, Kans., soil, 2; at Washington in greenhouse soil, 3; in sterilized soil infected with decayed beets, 2; in Madison greenhouse soil, 1; at Madison in

field soil, 1; crown-rot sugar beets from Rocky Ford, Colo., 5; from Garden City, Kans., 2; from Chino, Cal., 1; from Kenosha, Wis., 1; potato tuber from Carbondale, Colo., 1; radish from Madison, 1; carrot from Madison, 1; pine seedlings grown at Garden City (contributed by Mr. Carl Hartley, of the Bureau of Plant Industry), 2; and decaying tomato grown on Potomac Flats, Washington, D. C. (contributed by Dr. H. W. Wollenweber (44), of the Bureau of Plant Industry, as *R. potomacensis* Wollenw.), 1.

The various strains in cultures exhibited no striking differences. Those which did appear are due largely to difference in vigor. The virulence is reduced temporarily by long continuance in artificial culture. Difference in the virulence of the several strains, both when freshly isolated and when rejuvenated, was sometimes noted, and this difference appeared to be quite constant, although it bore no relation to the host which furnished the original culture. For example, one of the two strains most virulent to beet seedlings was secured from the beet root and the other was the form from tomatoes received as *R. potomacensis* Wollenw. Certain of the strains that were least virulent were obtained originally from sugar-beet seedlings.

The inoculations were carried out with extreme care, following the methods already described. Every precaution was taken to insure the accuracy of the results, which were uniformly positive. Each strain was recovered and reinoculated into seedlings through from four to six generations.

The type of disease produced upon beet seedlings is similar to that caused by *Phoma betae*, but the plants are attacked at a younger stage, and the progress of decay is likely to be more rapid, so that it was necessary to exercise considerable care in making inoculations at the time of seeding. In the cases of heavy inoculation few or no seedlings broke through the soil. With lighter inoculation a milder form of damping-off developed, or the disease took the form of root sickness, in which case a relatively large number of plants eventually recovered. The fungus is capable of attacking its host at any time after germination. Inoculations upon older seedlings also gave positive results. Young beets 4 or 5 weeks old were readily killed by inoculations upon the crown when no wound was made.

DISTRIBUTION OF THE FUNGUS

The distribution of the fungus is very general, but under field conditions damping-off due to Rhizoctonia is far more general in the soils of the semiarid West. Soils brought from western Kansas and Colorado to Wisconsin and placed in pots in the pathological garden yielded a large percentage of damping-off from *Rhizoctonia* sp., while Wisconsin soils in control pots were practically free from the ravages of this parasite. The fungus has been isolated a few times from the Wisconsin beet fields, but it appears to be of little consequence as a beet parasite under Wisconsin conditions. The reverse is true in Colorado and Kansas, where a majority

of the diseased seedlings examined have yielded cultures of Rhizoctonia. The fungus is the cause of a very destructive crown-rot in the West (Pl. XX, XXI, and XXII), where it frequently becomes epidemic. It is not uncommon to see entire fields of 50 or 100 acres practically destroyed in August by root-rot, of which there is no evidence earlier in the season (Pl. XXI, fig. 1). This form of rot is seen only occasionally in the more eastern beet-growing districts, where it appears to be of no economic importance.

CONDITIONS INFLUENCING INFECTION

The controlling influences in the distribution of the Rhizoctonia diseases of the beet may conceivably be associated with the unequal distribution of the fungus or with differences in climate or in soil, or with any combination of these. Some light has been shed upon this point in the course of the inoculation experiments on growing beets. Field inoculations were first made in Wisconsin, using cultures obtained from Colorado and Kansas. The first series was made on August 21, 1912, by placing portions of mycelium upon agar among the heart leaves of beets in the field. The inoculations were made just at dark, and the beet leaves were moistened with water from a sprinkler, in imitation of a heavy dew. The morning of the 22d was cloudy, and a very little rain fell. The weather of the next few days was dry and hot. Examination a few days later showed that infection had occurred in all except one of the 29 beets inoculated. The disease, however, failed to make the progress typical of western conditions. At the time of harvest, October 23, one beet showed no evidence of infection even at the point where inoculation was made and where the original dried culture was clearly seen. Six showed no injury other than slight lesions on petioles such as shown in Plate XXI, figure 2. Five showed old lesions on the crown, but they had entirely recovered. Eleven beets showed so slight evidence of decay that it was observed only on close examination. Five showed clearly defined decayed spots, but even these were restricted in area. One had entirely lost its original crown of leaves, but had formed scar tissue and had developed new leaves from the meristem at the sides. (Pl. XXII, fig. 1.) This was the only beet which had been injured for the commercial market, unless it might be that the sugar content of the others had been lowered.

A second set of inoculations were made on August 28. In this instance 40 beets were inoculated by placing on the crown of each a portion of Rhizoctonia mycelium growing on a sterilized beet block. The results were very much like those of the first series. While the inoculations took in every case, most of the beets outgrew the infection. Five beets were sufficiently injured to be unfit for market, but only one was killed. In these cases of serious infection the progress of the disease corresponds closely to that seen in the West, although it was far

less rapid. The fungus was readily recovered in culture from them. A photograph of the most seriously injured beet taken at the time of harvest on October 23 is reproduced in Plate XXII, figure 2. At that time the scars where the original infection had been produced could be found on all the beets. Some showed small areas of decay, but most of them were practically sound.

A third series of inoculations were made on September 11 through knife wounds near the surface of the ground. These were made in imitation of cultivator injury and were infected by placing a rapidly growing culture of *Rhizoctonia* sp. on a beet block directly in contact with the injured surface. Thirty beets were inoculated; none of them was destroyed. About half of the number healed completely, so as to leave only a local scar at the point of inoculation. The others showed local decay, more or less characteristic of crown-rot. The fungus was readily recovered from several of these. The largest decayed area produced was about 4 inches in diameter. At least half of this beet was still sound.

At the time of harvest the beets which showed no decay were topped to remove the leaves, the crown being left uninjured. They were placed on racks in the vegetable cellar and examined for decay from time to time. On April 3 all but two of them showed evidence of rot, although in most cases a close examination was necessary to discover it. Eighteen were selected and submitted to cultural tests for Rhizoctonia. Out of 68 attempts to isolate the organism 54 yielded *Phoma betae*, 2 failed to develop, and the remaining 12 gave growths of various saprophytes. In no instance was it possible to secure a culture of Rhizoctonia. It was apparent that those beets which failed to develop decay in the fields had entirely thrown off the infection from Rhizoctonia.

In order to determine to what extent this resistance to attack is to be attributed to local conditions of climate or soil, two large lots of soil from seriously infected beet fields, one in Kansas and one in Colorado, were shipped to Madison, Wis. Both types of soil were quite heavily infected with *Rhizoctonia* sp. That from Kansas was a sandy loam deficient in organic matter. It had received generous applications of factory waste lime and was of good mechanical texture. The Colorado soil was of compact structure containing an admixture of clay and fine silt. It was very deficient in organic matter, so that it was quite impervious and lumped badly. These soils were placed in unperforated, unglazed, 12-inch crocks containing cinders at the bottom for drainage, and sunk into the ground out of doors to within 2 inches of the top. Soil from Madison was employed in similar crocks as a control. Six crocks of each soil were sterilized in an autoclave by heating for 12 consecutive hours under 15 pounds' pressure, and six were left untreated. Untreated beet seed which showed remarkably strong germination and less than 1 per cent of infection with *Phoma betae* was sown. Damping-off developed only in the unsterilized soil from Kansas and Colorado. Attempts to

isolate the causal organisms in 27 cases gave the following results: Rhizoctonia, 15; Phoma, 8; Fusarium, 2; Mucor, 1; undetermined, 1. Both of the parasitic forms were isolated from each of the two types of soil developing disease. A good stand, which was thinned to three, four, or, in a few cases, five plants per pot, was secured in each crock, however, in spite of damping-off. Early in July evidences of crown-rot developed in four of the six pots of unsterilized Colorado soil, but not in that from Kansas. In two of these pots the stand was entirely destroyed by July 21, and in a third there remained only one small seedling with four leaves, which appeared after the original stand had been killed. No disease appeared in the pots of sterilized soil, nor did root-rot develop in the unsterilized Kansas soil. On July 23 inoculations were made with a recently isolated Kansas strain of Rhizoctonia in two pots of each of the six classes of soils. Two pots of each class were reserved as controls, and two were inoculated with *Phoma betæ*, as previously reported. The inoculations were made on one beet only in each pot, by placing a piece of beet-block culture on the crown and a second fragment against a wound just below the surface of the soil. The other beets in the pots were not disturbed in any way. One beet in each control pot was wounded in a manner similar to that employed in the inoculations.

The results in inoculated and in uninoculated pots are given in Table V. It is worthy of note that as a result of inoculating 1 beet in each of 12 pots, 26 beets were killed and 7 more were so seriously diseased as to be made worthless, while only 3 resisted infection. One beet attacked in July by spontaneous rot recovered later. The fungus was recovered in culture from several of the diseased roots.

TABLE V.—*Results of pot experiments with Rhizoctonia rot*

INOCULATED POTS

Source of soil.	Pot No.	Number of beets in pot on July 21.	Condition on October 18.		
			Number dead.	Number infected but living.	Number sound.
Colorado	19	3	3		
Do	22	[a] 1		1	
Colorado (sterilized)	1	4	3		1
Do	4	4	2	2	
Kansas	25	3	2	1	
Do	28	4	2	1	1
Kansas (sterilized)	31	2	2		
Do	34	4	4		
Wisconsin	13	2	1	1	
Do	16	3	1	1	1
Wisconsin (sterilized)	7	3	3		
Do	10	3	3		
Total		36	26	7	3

[a] The original stand of three beets had already been destroyed by spontaneous Rhizoctonia rot. The one plant living resulted from seed delayed in germination.

TABLE V.—*Results of pot experiments with Rhizoctonia rot*—Continued

UNINOCULATED POTS

Source of soil.	Pot No.	Number of beets in pot on July 21.	Condition on October 18.		
			Number dead.	Number infected but living.	Number sound.
Colorado	20				
Do	23	[a] 5	1		[b] 5
Colorado (sterilized)	2	4			4
Do	5	4			5
Kansas	26	3			3
Do	29	3			3
Kansas (sterilized)	32	2			2
Do	35	3			3
Wisconsin	14	4			4
Do	17	3			[b] 4
Wisconsin (sterilized)	8	3			3
Do	11	3			3
Total		37	1		39

[a] Two of these beets were diseased with Rhizoctonia rot. One of them eventually recovered, and the other died.

[b] The additional beet is known to have resulted from seed delayed in germination.

Other inoculations with Rhizoctonia were made on beets growing in the field at Madison, Wis., Garden City, Kans., and Rocky Ford, Colo.

Of 30 plants inoculated at Madison on July 23, 1 escaped infection, 2 were infected but recovered, and 27 were killed. The fungus was recovered in culture from several of them. The control plants, of which there were several hundred, remained healthy.

The inoculations at Garden City were made on July 27 on beets furnished by Dr. C. F. Clark, of the Bureau of Plant Industry, who kindly made the field observations. The field was known to be somewhat infected with Rhizoctonia. One row was inoculated, those adjacent on either side being reserved as controls. The same procedure was observed in the inoculations with *Phoma betae*, previously reported, but since these did not produce disease, five rows, or 150 beets, became available for controls. Of the 30 inoculated plants, 23 were killed, 2 others were so seriously injured at the crown as to become entirely defoliated and apparently dead but developed a few new leaves late in the fall (October), and 5 escaped infection. The rate of progress of the disease is shown in Table VI. Three control beets became infected during the season.

TABLE VI.—*Results of inoculation experiments with Rhizoctonia sp. at Garden City, Kans.*

Row No.	Treatment.	Number of dead sugar-beet plants on—						Total number of dead plants.	Number of healthy plants on Sept. 23.
		Aug. 7.	Aug. 13.	Aug. 23.	Sept. 3.	Sept. 17.	Sept. 23.		
1	Control.....	0	0	1	0	0	0	1	29
2	Rhizoctonia.....	2	1	11	6	1	4	[a] 25	5
3	Control.....	0	0	0	0	0	1	1	29
4	Do.....	1	0	0	0	0	0	1	29
5	*Phoma betae*.....	0	0	0	0	0	0	0	30
6	Control.....	0	0	0	0	0	0	0	30

[a] Two of these made feeble effort at recovery in October, showing that a little parenchyma had survived.

The 30 beets inoculated at Rocky Ford were killed, while all the controls remained healthy (Pl. XX, fig. 1).

A consideration of the facts related indicates that soil properties are potent factors influencing the susceptibility of beets to attack by Rhizoctonia. It has long been maintained that clay soils and those which are seriously deficient in organic matter and of fine, compact texture, so as to bake readily, are most likely to develop Rhizoctonia diseases. Further indication of this is found in the development of spontaneous root-rot in the Colorado soils used in the pot experiments at Wisconsin. It did not develop in the other soils employed, although the amount of Rhizoctonia damping-off indicated that the Kansas soil was at least as heavily infected with Rhizoctonia as was the Colorado material. The experimental data lead to the conclusion, however, that, in the case of the Rhizoctonia root-rot of the beet, soil temperature is a more important factor than soil texture. The inoculations in the field at Madison in 1912 were made at the beginning of a very hot period which endured for several days. Infection was produced uniformly, but in practically every case the beets completely recovered during the cooler weather which set in a few days after the inoculations were made. In 1913 infection was attempted at an earlier date when it might be expected that a somewhat longer period of hot weather would ensue. This proved to be the case and, as already pointed out, the inoculations were very generally successful. It is also significant that the cases of partial or complete recovery which occur appear late in the season when the soil temperatures are considerably lowered.

PYTHIUM DEBARYANUM

Hesse (24) reported *Pythium debaryanum* Hesse as a damping-off parasite of beets in 1874, but his experimental work appears to have been done on other hosts, so that while no one has doubted the accuracy of Hesse's deductions, Peters (33, p. 221) appears to have been the first to demonstrate by culture methods that this fungus includes the sugar

beet among its hosts. The fungus is so well known that a description here is superfluous. It is very readily secured in pure culture and is easily carried upon media (Pl. XVI, fig. 1). It grows especially well with long-continued vitality upon string-bean agar. The sexual fruiting bodies are quite common in Petri-dish cultures upon this medium, but are rarely met with in tube cultures. The asexual conidia, as well as oospores, are formed abundantly when the fungus is grown in water upon sugar-beet seedlings in Petri dishes. The cultures obtained throughout the experiments were invariably identified by fruiting bodies, and the same method was applied in proving up the cultures recovered from artificial inoculation. Suspected seedlings were treated in bichlorid of mercury, rinsed in water, and plated upon the acid synthetic agar previously mentioned (p. 137). When growth developed, the mycelium was examined through the bottom of the Petri dish by inverting the plate upon the stage of the microscope. If no septa were visible, the seedling was transferred to a sterile Petri dish after a subculture had been made from the growth. Sterile water was added to the fresh plate containing the seedling. In case the growth was *Pythium debaryanum*, the characteristic conidia developed in great numbers in from 24 to 48 hours, to be followed during the next few days by oospores. Direct germination of conidia was often seen and could be very readily induced by adding a fresh beet seedling to the culture. Germination by zoospores was not observed, but no special effort was made to induce this type of development.

The cultures used in the inoculation experiments were all morphologically identical, so far as could be determined. They were secured from the following sources:

Damped-off beets grown at Washington in the greenhouse, 2; at Madison, Wis., in the greenhouse, 6; at Madison in the greenhouse in Utah soil, 3; at Madison in the greenhouse in Michigan soil, 2; damped-off seedlings grown in Utah in the field, 1; grown in Wisconsin in the field, 1; grown in Colorado in the field, 1; damped-off pine seedlings from Kansas (contributed by Mr. Carl Hartley, of the Bureau of Plant Industry), 1; decaying potatoes, isolated in 1909, 1.

Mr. Hartley reported this strain pathogenic to pine seedlings, having produced damping-off with it to the extent of 100 per cent in the seed bed.

Pythium debaryanum proved to be exceedingly destructive in the pot experiments. When infection was made at the time of seeding, even a temporary stand was seldom secured. Examination showed that the seed germinated, but that the plants were destroyed before they could come up. In many cases the embryo was killed while still within the seed. By delaying the inoculation until the seedlings were well started typical damping-off was produced and the fungus recovered. It was a very common thing to find infection on the tips of the cotyledons.

This probably occurred while the leaves were still within the seed coat. The fungus was found to be capable of attacking the beet after it was 5 or 6 weeks old. Peters's statement (33, p. 228) that it is able to infect the side roots during the entire vegetative period is probably correct. When the taproot is once attacked by *P. debaryanum*, the ultimate death of the plant seems to be assured. Fortunately the soil relations in early seeding time are usually not sufficiently favorable to the rapid development of the fungus to make it an aggressive parasite under average field conditions. This fungus does not develop well in cold soil, but does its most serious work under seed-bed and greenhouse conditions or in the fields which have been seeded very late when the soil temperatures have begun to rise.

UNDESCRIBED SPECIES INJURIOUS TO SUGAR BEETS

In the author's preliminary note (12) it was reported that *Aphanomyces laevis* De By. had been found as a damping-off fungus of sugar beets in America, but subsequent detailed morphological studies of the fungus as it developed in artificial culture and on beet seedlings have shown that it differs in some important respects from the published descriptions of De Bary and others. *A. laevis* was first reported in a parasitic relation by Peters (31) in 1906. He found the fungus as a damping-off parasite of considerable importance upon sugar beets in Germany. Barrett (4) has reported its occurrence in America as the cause of a disease of radishes. The first cultures of the fungus temporarily mistaken for *A. laevis* were secured from damped-off beet seedlings grown in soil which had previously produced the black-root disease of the radish, like those shown in Plate XXIV, figure 2. It was later obtained from soils at Madison, and from Kenosha, Wis., as well as from seedlings damping-off in soil which had been infected with fragments of a diseased radish obtained from Illinois. The causal relation of the organism to the radish disease as well as to damping-off of sugar-beet seedlings was confirmed repeatedly by inoculation experiments, and it was at first thought possible that the discrepancies between this fungus and published descriptions of *A. laevis* might be the result of response to the changed environmental conditions of culture or to variations within the species, since it is well known from the study of many investigators that the Saprolegniaceae are exceedingly variable. Through the courtesy of the Kaiserliche Biologische Anstalt at Dahlem, Germany, and Dr. Leo Peters, of that institution, the author was permitted to isolate Aphanomyces from the experimental fields of the Anstalt. An organism was secured from damped-off seedlings, which Dr. Peters identified as the organism with which he had worked and which conformed in every respect to De Bary's description of *A. laevis*. It was secured in pure culture, and its pathogenicity to beet seedlings was confirmed by inoculation experiments. Unfortunately the culture was lost

before it had been tested upon the radish and thereafter could not be secured again. The morphological studies, however, prove that the American fungus with which we have been working is not *A. laevis*, but a hitherto undescribed organism. Morphological, physiological, and cytological studies will be presented in another paper.

In the work with sugar-beet seedlings five strains of the organism were employed which were obtained from the following sources:

Seedling sugar beets grown at Madison, Wis., 2; at Kenosha, Wis., 1; in soil originally infected by radishes showing black-root, 2.

The disease which it produces on the sugar beet is very simliar to that caused by *Pythium debaryanum*. The fungus is even more aggressive as a parasite than Pythium (Pl. XXIV, fig. 1). When the inoculations were made at the time of seeding, it was unusual for plants to appear above-ground. The evidence obtained all goes to show that a seedling once attacked never recovers.

A disease of the side roots of growing beets was encountered during the course of the studies in soils which had been inoculated with artificial cultures of the fungus several months earlier. A photograph of a specimen is reproduced in Plate XXIII, figure 2. The fungus was readily isolated from diseased side roots of this beet and there appears to be no reason to doubt its causal relation to the trouble. Peters (33, p. 244) has quoted a similar disease of European beets caused by Aphanomyces.

Comparatively little is known regarding the range of distribution of the fungus. What appears to be the black-root of radish has been observed in the District of Columbia, Maryland, Virginia, Long Island, Illinois, and at several points in Wisconsin, and there is reason to believe that other workers have found it in various places, although no records of such observations have been published. The disease produced by this fungus is so similar to that reported by Barrett (4) that they are not readily distinguishable, and it may be that either of these organisms is responsible for the disease in any of the stations mentioned. The author does not consider that his results should be construed to throw doubt upon the accuracy of Barrett's observations, merely wishing to record a radish disease that is indistinguishable in external appearance from that produced by *Aphanomyces laevis*, but which is due to this hitherto undescribed parasite.

The fungus is readily isolated from beet seedlings by the method already described for *Pythium debaryanum*. In this case, however, the subcultures should be made to string-bean agar, upon which the organism produces a luxuriant growth. Sterilized beet seedlings in water in test tubes make an excellent medium for the cultivation of this fungus. The limit of vitality in culture has not been determined, but transfers made to string-bean agar on July 2 from beet-seedling water cultures made on February 12 developed a heavy growth overnight, showing not the least loss of vitality.

The vegetative stage of the fungus is strikingly like that of *P. debaryanum*, so that they can not be distinguished readily except by the fruiting bodies, which develop readily in water cultures in plates in from 24 to 48 hours. The zoospores develop first, to be followed somewhat later by the Pythium-like oospores. The asexual fruiting bodies are first noted as the swollen ends of hyphæ, which vary greatly in length and are characteristically somewhat branched. They average from 150 to 900μ in length or even more. When mature, these bodies discharge their contents in a spherical mass which cleaves in the course of 20 or 30 minutes, giving rise to numerous zoospores.

OTHER FUNGI FOUND ON SUGAR BEETS

In the course of the isolation work various other fungi were secured. Some of these were known saprophytes, while others, like Macrosporium, Mucor, and Botrytis, have sometimes been reported in parasitic relations, but gave negative results in our trials. Fusarium and Verticillium cultures were secured frequently, but inoculation experiments with these genera were deferred pending the completion of taxonomic work by other investigators.

There remains to be discussed a peculiar type of decay of growing beets and a root sickness of seedlings associated with *Rhizopus nigricans* Ehr. Cultures of this fungus were frequently isolated in the course of experimental work from seedlings. Specimens of mature beets affected by a peculiar light-brown decay were received in the laboratory from California during the campaign of 1910. The interior portion of these beets yielded a very large proportion of cultures of Rhizopus. The decay was very characteristic and unlike anything before seen. In the early stages the material was almost normal in appearance, except for the discoloration. It later assumed a somewhat flabby texture and developed pockets in the interior which were filled with a nearly colorless fluid rich in acetic acid, as was determined by the odor and by chemical tests.

In 1912 a somewhat similar trouble was reported from Colorado, and a visit was made to the field (Pl. XXV, fig. 1). The beets at that time were dead over considerable areas. Those most recently attacked showed the same light-brown color previously referred to (Pl. XXV, fig. 2), while those in the more advanced stages of decay presented various symptoms between the early condition and almost complete dissolution of all except the vascular tissue. Some, however, were apparently pickled in acetic acid, formed probably from the fermentation of the carbohydrate content. A very large number of attempts to isolate an organism of known pathogenic properties was made. The trials yielded *Rhizopus nigricans* in pure cultures to the extent of almost 100 per cent. Inoculation work in the laboratory upon dormant beets in moist chambers resulted in the

reproduction of decay similar in appearance to that occurring in the two cases described. The acetic-acid development, however, did not occur except in a slight degree in a few instances, and there is no certainty that in these cases it did not result from contamination. Inoculation experiments made in the field upon living material invariably yielded negative results. Inoculation experiments upon seedlings made in the usual manner also failed to produce damping-off when reasonably good conditions of culture were maintained. It was possible, however, to produce a disease which showed the symptoms of root sickness when the soil was excessively wet and the temperature rather adverse. Naturally the fungus was easily isolated from such material. The control plants, however, were sickly or diseased on the roots, and it is highly probable that the results obtained in the inoculation experiments with seedlings are to be attributed to physiological injury, which opened the way for the Rhizopus to grow saprophytically upon the tissue.

Inquiry into the history of the fields that produced the peculiar rot with which this fungus was associated revealed the fact that at least one of them had been flooded for a time and that the other had been excessively moist for several consecutive days prior to the appearance of the disease. In view of these facts and the results of experimental work, it seems reasonable to conclude that the beets were originally killed or at least materially weakened by adverse physiological conditions and that *Rhizopus nigricans* followed as a saprophyte or weakling parasite producing a characteristic type of decay.

ALKALI INJURY TO SUGAR BEETS

During a field trip in Colorado late in August, 1912, the writer was called upon to visit a beet field in which a peculiar rot was developing. The beets had made a good growth, and most of them were above the average in size. The foliage was luxuriant, but was characterized by a bluish green color and a brittle texture. Little evidence of disease, aside from the abnormal appearance of the foliage, was evident until the plants were pulled, when it was seen that many beets were decayed at the lower portion of the root. Some agency had killed the taproot, following which a soft rot was destroying the tissue. A majority of the plants in the portions of the field most seriously affected showed a characteristic cracking and corroding of the root, like that shown in Plate XXVI.

Evidences of alkali could be seen on the surface of the soil here and there, and it seemed probable that the deeper branches of the taproot had been killed by alkaline waters. This probability was increased by the fact of the close proximity to the field of an irrigation reservoir the waters of which were evidently quite alkaline, as could be seen from the crust of salts on the ground at the edge of the lake. As a further test upon this opinion, several beets were taken to the laboratory and attempts were

made to isolate pathogenic organisms, but with negative results. Several of the plants upon which decay already had made considerable progress were placed in fresh soil in the greenhouse and held under observation to determine whether the disease would continue to develop. Without exception these plants healed and thereafter showed no evidences of disease. The foliage which they put forth was normal, giving no evidence of the brittleness or blue-green color noted in the field. The results seemed to justify the conclusion that the plants were suffering from excessive alkali brought in by seepage from the neighboring reservoir.

SUMMARY

The more important points brought out in this paper may be summarized as follows: Four fungi have been found to stand in a causal relation to damping-off of sugar beets in America. These are *Phoma betae* (Oud.) Fr.; *Rhizoctonia* sp. probably identical with *Corticium vagum* B. and C., var. *solani* Burt.; *Pythium debaryanum* Hesse; and an undescribed member of the Saprolegneaceae.

Under favorable conditions of culture, plants attacked by *Phoma betae* or Rhizoctonia may recover either temporarily or permanently. Attacks of the other two fungi upon the seedlings may be expected to prove fatal. Phoma and Rhizoctonia are capable of producing characteristic decay in mature beets. The former appears to infect the plants primarily in the seedling stage, and when recovery occurs it remains thereafter in a dormant condition upon the host. It occasionally develops a characteristic black rot on growing beets in the field and more frequently appears upon mother beets in storage. When it does not destroy the root, it may infect the seed stalk and appear upon the mature seed. Control measures are to be sought in proper cultural methods and seed treatment which looks forward to the production of seed free from infection. *Pythium debaryanum* is capable of attacking the feeding roots of the beet throughout its vegetative period, and the new fungus is also able to cause trouble on mature beets in a similar manner. *Rhizopus nigricans* Ehr., while unable to produce disease on normal plants in the field, is capable of attacking the tissue of dead or dormant sugar beets, producing a characteristic decay.

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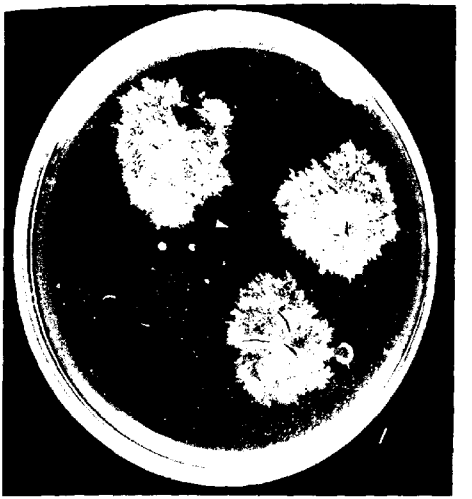
PLATE XVI

Isolation cultures from sugar-beet seedlings.

Fig. 1.—*Rhizoctonia* sp.
Fig. 2.—*Pythium debaryanum.*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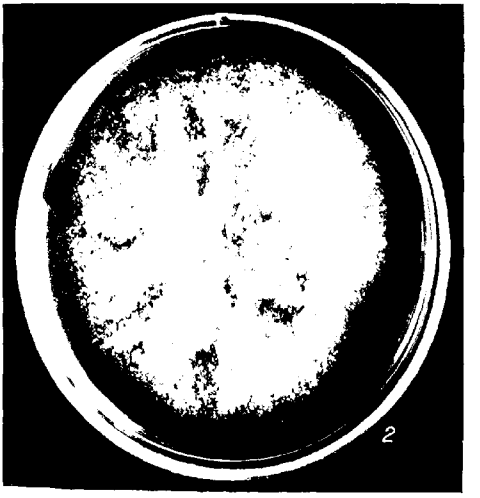
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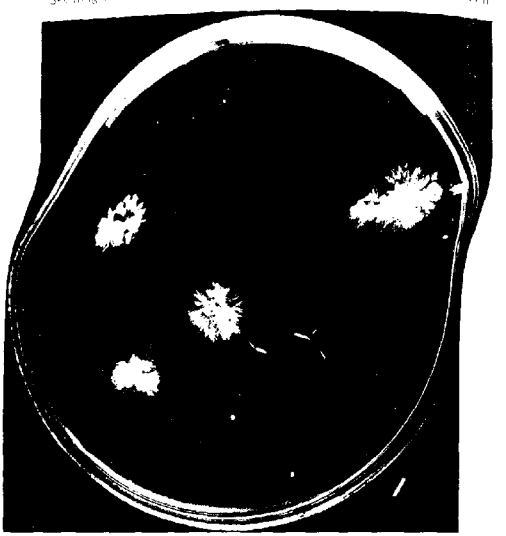


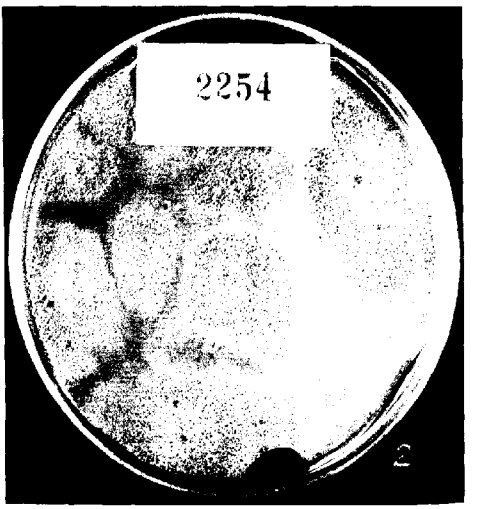

2254

PLATE XVII

Phoma betae.

Fig. 1.—Isolation culture from sugar-beet seedling.
Fig. 2.—Fruiting culture on string-bean agar.

PLATE XVIII

Fig. 1.—Half-grown sugar beets showing crown-rot caused by *Phoma betæ*.
Fig. 2.—Sugar beet showing seedling injury caused by *Phoma betæ*.

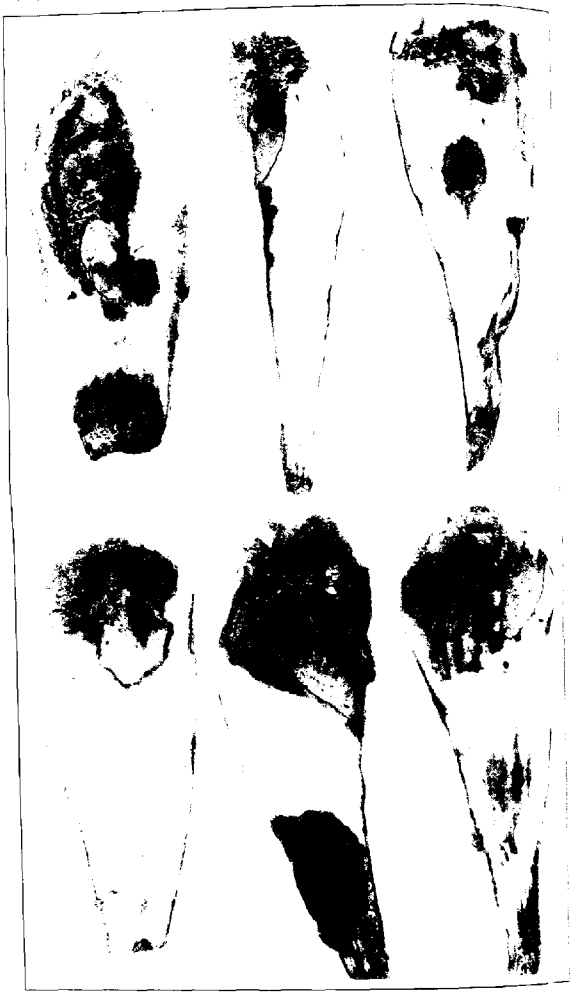


PLATE XIX

Mother beet showing storage decay caused by *Phoma betae*.

PLATE XX

Rhizoctonia sp.: Root-rot of sugar beet.

Fig. 1.—Result of artificial inoculation, control beet in center.
Fig. 2.—Result of natural field infection. Note sclerotia on specimens at right.


2


2

PLATE XXI

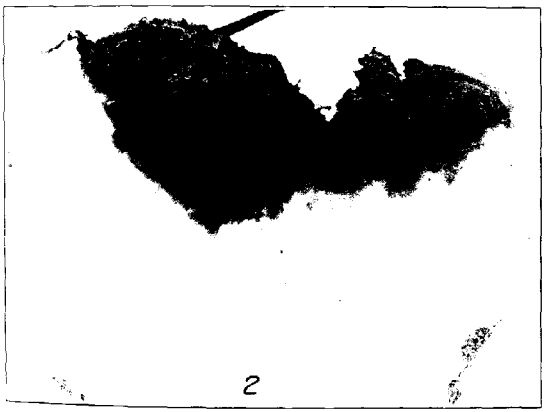
Fig. 1.—Sugar beet showing field-rot caused by *Rhizoctonia* sp. Natural infection.
Fig. 2. - Sugar beet showing artificial infection with *Rhizoctonia* sp. on the petiole. The disease has been arrested.

PLATE XXII

Results of artificial inoculation with *Rhizoctonia* sp.

Fig. 1.—Sugar beet, photographed from above, showing original crown destroyed, and new leaves developing from the sides.

Fig. 2.—Section of sugar beet showing character of Rhizoctonia injury.


2


2
1

PLATE XXIII

Fig. 1.—Sugar beet showing large sclerotium (½ inch) resulting from artificial inoculation with Rhizoctonia. The beet has resisted infection.

Fig. 2.—Half-grown sugar beet showing injury to feeding roots due to an undescribed parasite.

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PLATE XXIV

Fig. 1.—Sugar beet showing damping-off due to an undescribed parasite; control pot at right.

Fig. 2.—Radish showing black-root caused by the same fungus.


2


2

PLATE XXV

Fig. 1.—Field in which Rhizopus rot developed.
Fig. 2.—Typical beets from the field shown in figure 1.

PLATE XXVI

Sugar beet showing alkali injury.

PHOMA BETAE ON THE LEAVES OF THE SUGAR BEET

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INTRODUCTION

Various names have been given at different times to the fungus causing a root-rot, a damping-off, and a leaf-spot of the sugar beet (*Beta vulgaris* L.), and consequently their relationship to each other has not been recognized. The leaf-spotting was attributed by Oudemans[1] to *Phyllosticta betae* and later by Prillieux and Delacroix[2] to *P. tabifica*. Frank[3] believed the latter organism to be identical with his root-rot fungus, *Phoma betae*, but on account of generic differences no combination of names was made. Hedgcock[4] pointed out for the first time a definite connection between Phyllosticta on the leaf and Phoma on the root. Peters[5] and Edson[6] give evidence that the fungus which produces leaf-spotting is also a cause of damping-off. The present investigation shows that the leaf-spot and the root-rot organism are the same and points out that the entire life cycle of the fungus must be considered in any interpretation that is made of the disease phenomena. The name "*Phoma betae* (Oud.) Fr." is deemed by the writers and by Edson[6] to be correct and inclusive; however, the generic name "Phyllosticta" is retained in this paper for the organism isolated from leaves.

SYMPTOMATOLOGY

A mature, normally developed spot of *Phoma betae* on the sugar-beet leaf varies in size from 1 to 2 cm. in diameter and is usually light brown in color. At times such spots show concentric rings of growth, the different zones being outlined by pycnidia. There is no sharp differentiation (Pl. XXVII) between the infected area and the surrounding tissue, owing to the lessened activity of the beet leaf at the time the organism is growing in the leaf tissue. This accounts for the comparatively large size of the spot and its rather diffusive character. The spots which

[1] Oudemans, C. A. J. A. Aanwinsten voor de flora mycologica van Nederland van Juli 1875 tot Juli. 1876. *In* Nederland. Kruidk. Arch., s. 2, deel 2, stuk 3, p. 181. 1877.

[2] Prillieux, E. E., and Delacroix, Georges. Complément à l'étude de la maladie du cœur de la betterave. *In* Bul. Soc. Mycol. France, t. 7, p. 23–25, pl. 3. 1891.

[3] Frank, A. B. Phoma Betae, ein neuer Rübenpilz. *In* Ztschr. Pflanzenkrank., Bd. 3, p. 90–92. 1893.

[4] Hedgcock, G. G. Proof of the identity of Phoma and Phyllosticta on the sugar beet. *In* Jour. Mycol., v. 10, p. 2–3. 1904.

[5] Peters, Leo. Ueber die Erreger des Wurzelbrandes. *In* Arb. K. Biol. Anst. Land- u. Forstw., Bd. 8, Heft 2, p. 229–239. 1911.

[6] Edson, H. A. Seedling diseases of sugar beets and their relation to root-rot and crown-rot. *In* Jour Agr. Research, v. 4, no. 2, p. 135–168. 1915.

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first appear are generally brown, rarely red, in color. The latter color suggests that the lesion was first produced by some injury which probably caused the formation of carotin, the fungus later gaining an entrance. Typical spots grow rapidly and after 10 days or 2 weeks the black, somewhat erumpent pycnidia develop.

During the growing season of 1912 at Rocky Ford, Colo., observations were made of the different types of Phoma spots that were found to occur. Small brown spots were first noted on old mature leaves in the early part of July. Cultures made from such spots developed colonies of *Cercospora beticola* and *Phoma betae*. Several small red spots collected somewhat later gave either pure cultures of *P. betae* or a mixture of Phoma and an Alternaria. It would seem that the earliest spots contained more than one organism, owing probably to the fact that insect wounds made it easy for various fungi to enter. These spots frequently did not enlarge, showing that the organism had gained no sure foothold. By the last of July or the first part of August large, light-brown, typical spots yielded pure cultures of *P. betae*. Such spots always occurred on those leaves which were old and showed symptoms of yellowing. Consequently on a normal beet plant only a few leaves were infected, but on a plant that was physiologically weakened as a result of rot caused by *Rhizoctonia solani* or of some other factor inimical to plant growth many leaves were found to have typical spots. This observation was confirmed during the season of 1914 at Madison, Wis., where many of the leaves on the "mother beet" plants were found to be infected with Phoma. The roots from which these plants had grown had been more or less affected by various storage rots during the preceding winter, and consequently the vitality of the plants was greatly lowered.

The leaves attacked on the normal and abnormal plants showed the same symptoms of age. Thus, it would seem from field observations that the age of the leaf becomes the important factor in its susceptibility to the disease, and this is upheld in controlled experiments.

AGE AS A FACTOR IN LEAF SUSCEPTIBILITY

Practically all inoculation experiments carried on in 1912 to determine the connection between *Phoma betae* and *Phyllosticta betae* gave negative results. In all the preliminary studies the pycnospores of Phoma from the root and of Phyllosticta from the leaf were suspended in sterile water and either sprayed or smeared on leaves of all ages. Out of 150 inoculations thus made there were only four infections, and these occurred on old, yellow leaves, indicating that the organism is only rarely able to penetrate the unbroken epidermis. Later work has shown that even at the most favorable age the great majority of infections take place through some lesion on the leaf surface.

It was found, after making a large number of counts, that the maturity and the relative age of the different leaves of a beet plant could be determined by taking an average of the number of stomata on a given area at the base, middle, and apex of the leaf.[1] Numerous preliminary determinations showed that within certain ranges the number of stomata that occurred on either surface of the leaf was indicative of its age, so, for convenience, all subsequent counts were made on the upper surface. It was ascertained that leaves with 53 to 100 stomata per square milimeter could be considered as mature and were so designated. Every leaf in the outermost whorls on all plants examined gave stomatal counts within this range. Presumably the cells of such leaves had reached their maximum growth and their greatest metabolic activity. Young mature leaves which had a stomatal count per square milimeter of 92 to 133 were usually taken from a medium position on the plant and were metabolically active, although they had not as yet reached their greatest size. Leaves which had 134 or more stomata per square milimeter were very immature and were located near the heart growth of the plant.

In order to determine which were the most susceptible to infection by *Phoma betae*, 21 needle lesion inoculations on young leaves, 39 on medium-aged leaves, and 90 on old, mature leaves were made. Only 34 infections developed, and these were on the old, mature leaves. A comparable series of inoculations made with *Phyllosticta betae* gave 25 infections from 91 inoculations on old, mature leaves, no infections from 12 inoculations made on medium-aged leaves, and none from 42 made on young leaves. On the petioles 50 inoculations gave no infection with either organism. The number of infections was not increased when the plants were covered with bell jars or pots, but the infected areas appeared somewhat sooner than on uncovered plants. Typical spots developed, if at all, from two to four days after inoculating; however, this incubation time is in all probability lengthened under less favorable field conditions. (See Table I.)

[1] The stomatoscope originated by Prof. F. E. Lloyd was made available for the work through the kindness of the Alabama Polytechnic Institute. At times an adaptation of the stomatoscope with an ordinary microscope was employed.

TABLE I.—*Results of inoculating sugar-beet leaves of different ages* [a] *with Phoma betae and Phyllosticta betae*

Age of leaves and series No.	Phoma betae. Average number of stomata per sq. mm. of upper leaf surface.	Phoma betae. Number of inoculations per leaf.	Phoma betae. Condition of leaf four days after inoculation.	Phoma betae. Number of infections per leaf. [b]	Phyllosticta betae. Average number of stomata per sq. mm. of upper leaf surface.	Phyllosticta betae. Number of inoculations per leaf.	Phyllosticta betae. Condition of leaf four days after inoculation.	Phyllosticta betae. Number of infections per leaf.
Old leaves [c] (series 1).	62.8	3	Yellowing	2	71.0	3	Yellow	
	73.8	3	Old mature	3	60.1	3	Old mature	
	66.1	3	Yellowing	3	61.5	3	Old yellow	
	68.3	3	Beginning to yellow	3	71.0	3	do	
	79.2	3	Slightly yellowed	3	87.4	3	Mature	
	76.5	3	Yellowing	2	82.0	3	Yellowing	
	61.5	3	Old yellowing	3	90.2	3	Old mature	
	73.8	3	Yellowing	3	71.0	3	Beginning to yellow	
	60.1	3	do	3	90.2	3	Mature	
	100.0	3	Old mature	2	76.5	3	Old yellow	
	90.2	3	do	3	98.4	3	Mature	
	60.6	3	Beginning to yellow	3	76.5	3	Yellowing	
	82.0	3	Old mature	1	90.2	3	Old mature	
	68.3	3	do	0	87.4	3	do	
	85.2	3	Mature	0	71.0	3	Mature	
	88.1	3	do	0	60.0	3		
	82.0	3	do	0	82.0	3	Mature	
	98.4	3	do	0	71.0	3	Old mature	
	87.4	3	Old mature	0	84.7	3	Mature	
	84.0	3	do	0	95.6	3	do	
	82.0	3	do	0	82.0	3	Beginning to yellow	
	82.0	3	do	0	82.0	3	Mature	
	65.6	3	Mature	0	68.3	3	do	
	84.7	3	Old mature	0	73.8	3	do	
	82.0	3	do	0	92.9	3	do	
	86.9	3	Mature	0	86.1	3	do	
	82.0	3	Old mature	0	84.7	3	do	
	73.8	3	do	0	84.7	3	do	
	84.0	3	do	0	98.4	3	do	
	95.6	3	do	0	92.9	3	do	
					98.4	3	do	
					87.4	3	Beginning to yellow	
					79.2	3	Mature	
	Total	90		34		90		21
Old leaves [c] (series 2).	65.6	4	Slightly yellow	1	84.7	4	Yellow	
	60.1	4	Beginning to yellow and dying	3	87.4	4	Still green	
	82.0	4	Yellow	3	79.2	4	Slightly yellow	
	76.5	4	do	4	82.0	4	Still green	
	65.6	4	Yellow and dead	4	82.0	4	do	
	79.2	4	Beginning to yellow	1	71.0	4	do	
	84.7	4	Quite yellow	4	79.2	4	Rather more green than yellow	
	73.8	4	Yellowing	2	76.5	4	Yellowing	
	57.4	4	do	4	84.7	4	Yellow and dying	
	62.8	4	do	2	73.8	4	Still rather green	
	87.4	4	Still somewhat green	4	73.8	4	Yellow	
	87.4	4	Still green	0	73.8	4	do	
	Total	48		32		48		23
Young mature leaves [d] (series 3).	106.6	3		0	101.1	3		
	103.8	3		0	106.6	3		
	117.5	3		0	109.3	3		
	103.8	3		0	110.7	3		
	110.7	3		0				
	103.8	3		0				
	99.3	3		0				
	110.7	3		0				
	103.8	3		0				
	106.6	3		0				
	112.7	3		0				
	106.6	3		0				
	117.5	3		0				
	Total	39				12		

[a] Inoculations made on plants in the greenhouse of Bureau of Plant Industry, at Washington, D. C., on January 13 and 24, 1914.

[b] *Phoma betae* was reisolated from all infected spots that are indicated in series 1 and 2.

[c] The leaves in this series occupied the outermost position on the beet plants.

[d] The leaves designated as "young mature" were those occupying a medium position in the plant growth

TABLE I.—*Results of inoculating sugar-beet leaves of different ages with Phoma betae and Phyllosticta betae*—Continued

Age of leaves and series No.	Phoma betae.				Phyllosticta betae.			
	Average number of stomata per sq. mm. of upper leaf surface.	Number of inoculations per leaf.	Condition of leaf four days after inoculation.	Number of infections per leaf.	Average number of stomata per sq. mm. of upper leaf surface.	Number of inoculations per leaf.	Condition of leaf four days after inoculation.	Number of infections per leaf.
Very immature or "heart" leaves (series 4).[a]	142.1	3	.....	0	133.9	3	.....	0
	153.0	3	.....	0	174.9	3	.....	0
	152.5	3	.....	0	169.4	3	.....	0
	139.4	3	.....	0	143.5	3	.....	0
	147.6	3	.....	0	164.0	3	.....	0
	153.0	3	.....	0	169.4	3	.....	0
	155.8	3	.....	0	136.6	3	.....	0
					147.6	3	.....	0
					180.4	3	.....	0
					144.8	3	.....	0
					172.2	3	.....	0
					232.3	3	.....	0
					158.5	3	.....	0
					172.2	3	.....	0
	Total..	21	.....	.....	.....	52	.....	.....

[a] "Heart" leaves occupied the central portion of the plant.

The data recorded in Table I (series 1, 3, and 4) show that the old, mature leaves of the sugar-beet plant or those leaves which are beginning to yellow are the only ones that are susceptible to *Phoma betae*. In order to corroborate this point, additional inoculations were made on leaves that were deemed to be in this condition at the time of inoculating. Out of 48 inoculations with *Phoma betae* 32 typical spots were produced, and with a like number of inoculations with *Phyllosticta betae* 23 infections developed. It will be noted in Table I (series 2) that the inoculations which did not produce infections were made on leaves that were evidently not correctly determined as to their degree of maturity, since, as a rule, they had not yet attained this age at the time the infections should have developed. The cultures in this series were obtained from reisolations of Phoma and Phyllosticta infections of series 1.

The original cultures used for the inoculations with *Phoma betae* were obtained from Mr. H. A. Edson, of the Bureau of Plant Industry, who isolated the organism from rotted sugar beets kept in storage at Longmont, Colo. Those of *Phyllosticta betae* used in the inoculations were obtained from heavily infected sugar-beet leaves that had been collected in Colorado during the growing season of 1913. Puncture inoculations used exclusively in this experiment were made from a suspension of the pycnospores in sterile water.

DISSEMINATION OF PHOMA BETAE

Such agencies as beet balls,[1] wind, irrigation water, insects, and dung play an important part in the distribution of *Phoma betae* in the field.

[1] Edson, H. A. Op. cit., p. 141.

From 44 plate cultures exposed (2 plates at a time) for 15 to 30 minutes near the open ground surface in various beet fields at different times from June 7 to September 10, 1912, inclusive, at Rocky Ford, Colo., 50 colonies of *P. betae* were obtained. The fungus was present in the air at temperatures which varied from 68° to 115° F. at the ground surface, with relative humidities from 39 to 71 per cent. These readings were taken during the time that the plates were exposed. The organism was not obtained from plates exposed during one night, an experiment which was of necessity limited. Its presence in the air seemed to be dependent on the humidity, a high humidity apparently causing the pycnidia to expel their spores, while a subsequent decrease in the relative humidity caused the spores to escape into the air.

At certain times *P. betae* occurs abundantly in irrigation water. This is particularly true late in August and early in September. The pycnidia are well formed on the leaves by this time, and if moistened they burst and many spores are expelled. Samples of irrigation water which was either standing between the rows or had drained to the lower portions of the beet fields about one day after irrigation yielded Phoma in several cases in the tests made in 1912. Thirty-three colonies of *P. betae* in plate cultures were obtained from 23 c. c. of water representing four such samples, while 3 c. c. of water flowing through a field yielded nine colonies in cultures.

Three species of insects have been found to be carriers of the fungus to only a slight extent. Two culture tests made with the moth of the beet webworm, *Loxostege sticticalis* L., yielded many colonies of *P. betae* in the latter part of July, while cultures made at later intervals gave negative results. Several tests made of the alkali beetle, *Monoxia punctipollis* Say, and the larvæ of the woolly bear (yellow), *Diacrisia virginica* Fab., yielded only a few colonies of the fungus.

Phoma betae may occur in abundance in the dung present in feed yards where beet tops have been fed. It is not to be concluded that the presence of the organism here indicates that it can survive a passage through the alimentary tract of cattle or sheep, but rather that the fungus is viable in dung after the ordinary method of feeding beet tops where they are not entirely consumed. In one test made early in January, 1913, 36 colonies of *P. betae* were obtained from nine small drops of strong manure decoction.

FACTORS INIMICAL TO VIABILILITY OF PHOMA BETAE IN BEET LEAVES

DRY HEAT

The thermal death point of *Phoma betae* in sugar-beet leaf tissue exposed for half an hour to dry heat is between 80° and 90° C. Seventy isolations of *Phoma betae* were made from spots on leaves exposed at 70° for half an hour. At 80° two colonies developed in cultures made from approxi-

mately the same amount of material, and at 90° and 100° none were obtained. It is evident, therefore, that the fungus would be rendered harmless when infected beet tops are dried in a pulp drier.

OVERWINTERING UNDER FIELD CONDITIONS

Phoma betae has been found to be present to a slight extent in the soil of old sugar-beet fields. It was isolated from samples of finely divided soil taken during March and April, 1912, from fields near Rocky Ford, Colo., which had been in sugar beets for several years. Four colonies were obtained from 0.05 gm. of a surface sample, while from cultures made from 0.25 gm. representing two different first-foot samples eight colonies were obtained. No growth of the fungus occurred in cultures made from second- and third-foot samples. Although the tests were continued throughout May, June, July, and the first part of August, no further isolations were made.

About the middle of October, 1912, sugar-beet leaves which were infected with *P. betae* were mixed with soil to the depth of 6 inches in a box and left exposed to outdoor weather changes. No cultures of the organism could be obtained from these leaves after 3 months. However, different results were obtained when the leaves were buried at various depths in the ground. It was found that the fungus was viable at the end of 3 months in leaves which had been buried at depths of 1 to 5 inches or had been kept in the interior of a pile of hayed beet leaves. The organism was isolated from leaves buried at depths of 1 to 5 inches after 5 months, but there was no development from the leaves buried 6 to 8 inches. At the end of 12 months no growth of *P. betae* was obtained at any depth, and the leaves were practically all disintegrated. However, the viability of the fungus was not impaired in dried leaves stored under herbarium conditions for over 2 years.

The maximum temperature of the air from October, 1912, to September, 1913, inclusive, the time of the overwintering experiment, varied from 4° to 102°, the minimum from −20° to 72° F. The maximum temperature of the ground at a depth of 5 inches from December, 1912, to May, 1913, inclusive, varied from 42° to 92°, the minimum from 22° to 51° F. During the 12 months of the experiment there was 9.34 inches of rainfall and snow, mostly during April, May, June, and July. There was no precipitation during November and December of 1912, but there occurred 0.2 inch in January, and 0.4 inch in October, 1913. During this time the lowest soil and air temperatures were registered.

It appears, then, that the results on the viability of the organism obtained from covering the leaves with soil in boxes are not comparable to those obtained under field conditions. The temperature of the air varies from that of the soil to such a degree that accurate results for field comparison can not be obtained in such an experiment. A period of one year seems sufficient to eliminate *Phoma betae* from infected beet-leaf material left in the field, although there is a probability that the

fungus mycelium may remain dormant for a longer period of time in a sugar-beet root or "mother beet" stalk. The writers have found no evidence of a perfect stage of the organism.

The leaves for the outdoor-exposure experiments were buried in such a manner that examination was rendered convenient and accurate. The following method was suggested by Mr. W. A. Orton, Pathologist in Charge of Cotton- and Truck-Disease and Sugar-Plant Investigations, Bureau of Plant Industry. The soil was removed to the depth required and a piece of 2-inch mesh wire was placed on the exposed ground surface. The layer of leaves was then packed over this, another layer of wire added, and then soil to the depth desired. In examining the leaves at any time the layer of wire served to show the position of the leaves, and definite spots could be taken for cultural material. The effect of outdoor winter conditions on the viability of *Phoma betae* in infected beet tops is given in Table II.

TABLE II.—*Effect of different methods of overwintering on the viability of Phoma betae in infected beet leaves*

Method of treatment of infected leaves.	Length of exposure.	Number of cultures made.[a]	Number of isolations.[b]
	Months.		
Buried in soil in box	3	3	None.
Do	4	7	None.
Do	7	6	None.
Buried 1 inch in ground	6	21	Many.
Do	8	21	Few.
Do	10	4	Few.
Do	11½	8	None.
Buried 2 inches in ground	6	19	8.
Do	10	5	Few.
Do	11½	8	None.
Buried 3 inches in ground	6	21	Many.
Do	10	4	None.
Do	11½	8	None.
Buried 4 inches in ground	6	19	Many.
Do	10	6	None.
Do	11½	8	None.
Buried 5 inches in ground	6	20	Many.
Do	10	5	None.
Do	11½	8	None.
Buried 6 inches in ground	6	20	None.
Do	10	4	None.
Do	11½	8	None.
Buried 7 inches in ground	6	15	None.
Do	8	10	None.
Do	10	4	None.
Do	11½	8	None.
Buried 8 inches in ground	6	20	None.
Do	8	10	None.
Do	10	4	None.
Do	11½	8	None.
Interior of "hayed" pile of beet tops	3½	30	6.
Do	4	8	1.
Do	6	6	2.
Do	11½	8	None.
Left in field on surface of ground	5½	11	6.

[a] String-bean agar was used for all cultures.
[b] The number of spots used for each cultural plate varied from 1 to 4 or 5.

ENSILAGE

The process of siloing infected beet tops has been found to be sufficient to kill *Phoma betae*. In the ensilage experiments carried on during the winters of 1912 and 1913 it was ascertained that the organism was viable at the time the silage was made, but could not be isolated after the tops had been ensiled for two months. A medium composed of somewhat diluted silage material was also inimical to the growth of the fungus. Detailed data will be published later in connection with the relation of *Cercospora beticola* to ensiled beet tops.

SUMMARY

A typical spot of *Phoma betae* (Oud.) Fr. is light brown in color, 1 to 2 cm. in diameter, and has scattered over its surface numerous pycnidia, at times concentrically arranged. Such spots on a normal beet plant usually appear during July and August on the old leaves near the ground. If the plant is physiologically low, all except the heart leaves may become infected.

Phoma betae produces a characteristic infection on leaves that have a stomatal count of 60 to 100 per sq. mm. of upper leaf surface.

The pycnospores of the fungus may be disseminated by such agencies as beet balls, wind, irrigation water, insects, and dung.

The thermal death point of *Phoma betae* in the leaf tissue exposed for one-half hour to dry heat is between 80° and 90° C. The fungus is dead in infected leaves after three months' storage in soil in boxes exposed to outdoor conditions, while its life becomes extinct in leaves buried in the ground only after five to eight months, depending on the depth of cover. The fungus can not survive the process of ensiling the beet tops.

PLATE XXVII

An old leaf of a sugar-beet plant showing typical spots of *Phoma betae*.

NOTES ON THE HYDROCYANIC-ACID CONTENT OF SORGHUM

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INTRODUCTION

In the course of our work on the chemistry of *Sorghum vulgare* it was thought desirable to study its content of hydrocyanic acid (HCN) under Minnesota conditions. Many instances are on record of the poisoning of cattle from feeding on growing sorghum cane, and some of these cases have been definitely proved to be due to hydrocyanic acid, which occurs in sorghum as a constituent of the glucosid dhurrin (6).[1]

The factors which affect the amount of this glucosid in the plant have received some attention. All investigators have found that it decreases as the plant matures. Maxwell (8) states that sorghum is not fed with safety until after the seeds begin to develop; Brünnich (4), that it should not be fed until the seeds are fully matured. Avery (2) says that the amount of hydrocyanic acid is greater in stunted plants, while Alway and Trumbull (1) found that yellow, stunted plants contained less of the acid than the green, vigorous plants in the same field. Maxwell (8) believes that the amount of the glucosid is dependent on the character of the soil, soils rich in nitrogen producing plants richer in the glucosid. Brünnich (4), in experiments with sodium nitrate in Queensland, found that the fertilized plants contained slightly more hydrocyanic acid than those unfertilized and concluded that heavy nitrogenous soils and favorable climatic conditions increase the amount of the acid. His findings were corroborated by Alway and Trumbull (1). Brünnich (5) also found that millet (*Panicum miliaceum*) behaved similarly to sorghum. Schröder and Dammann (10), in Uruguay, report an increase in prussic acid due to the use of sodium nitrate as a fertilizer. Balfour (3) noticed that plants infected with *Aphis sorghi* contained more hydrocyanic acid than uninfected plants. These are the main facts which have been published in the literature concerning the occurrence of a cyanogenetic glucosid in sorghum.

Samples of the 1913 crop of cane grown on the farm of the University of Minnesota were analyzed about the middle of August, and the acid was found to be absent in all cases. As it has often been proved to persist in the plant to a later period, this result was considered unusual, and it was decided to repeat the work the next season. On analyzing a sample of plants 6 inches high, taken on June 26, 1914, hydrocyanic

[1] Reference is made by number to "Literature cited," p. 185.

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acid to the amount of 0.058 per cent of the dry matter was found. In view of the inconsistencies found in the above reports and in our analyses, it was deemed advisable to study the question further, especially with respect to the production and distribution of the prussic acid in sorghum.

EXPERIMENTAL WORK

Two of the plots of sorghum grown on the university farm in rich, fertile soil were selected, and the second row of each was treated with dried blood at the rate of 800 pounds per acre. This left five check

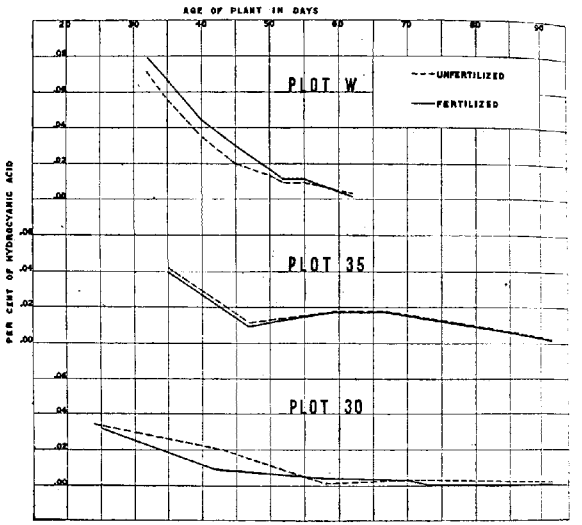

FIG. 1.—Curve showing the effect of available nitrogen on the hydrocyanic-acid content of sorghum. The percentage of hydrocyanic acid is based on dry matter.

rows unfertilized. The fertilizer was applied on July 2, when the plants were about 8 inches high. Samples of the crop were taken at intervals thereafter and analyzed for hydrocyanic acid. At the same time six rows of sorghum (Early Rose variety) were planted in some very poor, sandy soil. The first row was left as a check, the second treated with dried blood at the rate of 100 pounds per acre, the third with 200 pounds, the fourth left as a check, the fifth with 400 pounds, and the sixth with 800 pounds per acre.

The six rows planted on the sandy plot of ground thrived very poorly. They were slow in sprouting, owing to dry weather, and for the first few weeks grew very slowly. The soil was too poor to support plant growth

adequately, and after about eight weeks of slow growth the leaves turned yellow and frosts came before the plants were well headed out. This condition, together with the use of the fertilizer, gave us an opportunity to study the occurrence of prussic acid in poorly nourished plants and to compare them with those having a better supply of nitrogen.

In the samples from this plot the whole plant was ground up for the determination of hydrocyanic acid. In general, the cane in the rows which received fertilizer grew a little better than that which did not, but Table I shows that the increase in hydrocyanic acid is inappreciable. It can be detected only by comparing the average of the two check rows with the average of Rows V and VI, which received the heaviest applications of fertilizer. This comparison is shown in figure 1, plot W, the dotted lines representing the average of Rows I and IV and the solid line that of Rows V and VI. In a measure these results substantiate the work of some of the investigators mentioned above, in that the soils with the better supply of nitrogen were found to have produced plants with a higher content of hydrocyanic acid. The difference, however, is very slight, and the findings of Alway and Trumbull (1) rather than those of Avery (2) are supported, for the reason that the stunted plants showed less hydrocyanic acid than the thrifty ones. In these plots the amount of the acid in the early stages was higher than in the plots having good soil (fig. 1, plots 30 and 35), but it persisted through a much shorter period of the plant's life.

TABLE I.—*Effect of available nitrogen on the hydrocyanic-acid content of sorghum*

[Percentage of hydrocyanic acid is reported on a dry-matter basis]

PLOT W

Row or plot and sample number.	Height.	Age.	Percentage of hydrocyanic acid.		
			Stalks.	Leaves.	Whole plant.
Row I:[a]	*Inches.*	*Days.*			
1	18	31			0.083
2	28	38			.032
3	39	52			.000
4	42	55			.007
5	61	62			.002
Row II:[b]					
6	19	31			.083
7	31	38			.027
8	40	45			.025
9	57	62			.000
Row III:[c]					
10	20	31			.083
11	34	38			.022
12	40	45			.032
13	56	62			.005
Row IV:[a]					
14	26	32			.064
15	34	39			.043
16	40	45			.024
17	56	62			.007

[a] Check. [b] Fertilized at rate of 100 pounds per acre. [c] Fertilized at rate of 200 pounds per acre.

TABLE I.—*Effect of available nitrogen on the hydrocyanic-acid content of sorghum*—Con.

PLOT W—continued

Row or plot and sample number.	Height.	Age.	Percentage of hydrocyanic acid.		
			Stalks.	Leaves.	Whole plant.
	Inches.	*Days.*			
Row V:[a]					
18	26	32			0.068
19	38	39			.045
20	42	52			.021
21	59	62			.009
Row VI:[b]					
22	21	32			.095
23	32	39			.049
24	43	52			.009
25	40	55			.007
26	58	62			.003

PLOTS 35 AND 30

Plot 35 (feterita):[c]					
27	22	24	0.077	0.025	
28	39	35	.053	.032	0.041
29	57	47	.010	.013	.011
30	77	59	.0068	.037	.017
31	90	67	.0009	.033	.017
32	83	92	.0000	.008	.0021
Plot 35 (feterita):[b]					
33	22	24	.077	.052	
34	36	35	.065	.027	.040
35	60	47	.0047	.015	.009
36	81	59	.0068	.037	.017
37	89	67	.0068	.034	.017
38	85	92	.0000	.018	.0035
Plot 30 (Orange sorgo):[c]					
39	29	25	.050	.019	.033
40	59	43	.000	.042	.020
41	102	58	.000	.005	.0011
42	103	70	.000	.022	.0034
43	100	73	.000	.021	.0022
44	96	92	.000	.0065	.0013
Plot 30 (Orange sorgo):[b]					
45	28	25	.050	.017	.032
46	60	42	.000	.019	.0093
47	96	58	.000	.014	.0038
48	105	70	.000	.016	.0027
49	95	73	.000	.0036	.0005
50	97	92	.000	.0046	.0007

[a] Fertilized at rate of 400 pounds per acre. [b] Fertilized at rate of 800 pounds per acre. [c] Check.

In Table I are also brought together the analyses of the samples from the plots on the fertile ground on the university farm. Plot 35 was feterita, a variety of sorghum; plot 30 was Orange sorgo. In these experiments the leaves were stripped from the stalks, the hydrocyanic acid determined on each portion, and the percentage of the acid in the whole plant calculated on the basis of the relative proportion of dry matter in the leaves and stalks. In figure 1 the results for the whole plant are

shown graphically. Here, again, there appears only a slight difference in the hydrocyanic-acid content due to the added nitrogen fertilizer; but contrary to the effect shown in Table I and the curve for plot W, the slightly lower percentage of prussic acid was found in the plants from the fertilized row. In plot 35 the decrease is insignificant, and in plot 30 the two curves cross each other twice. Taking into consideration the results from all three plots, it appears that on soils deficient in nitrogen added nitrogen will slightly increase the prussic acid in sorghum; but that with a plentiful supply of nutrients in the soil added nitrogen does not affect the amount of the acid in the plants.

A plentiful supply of nitrogen in the soil will permit the maintenance of a definite amount of prussic acid at a given stage of growth; but it may be that this amount is not absolutely required and that if the supply of

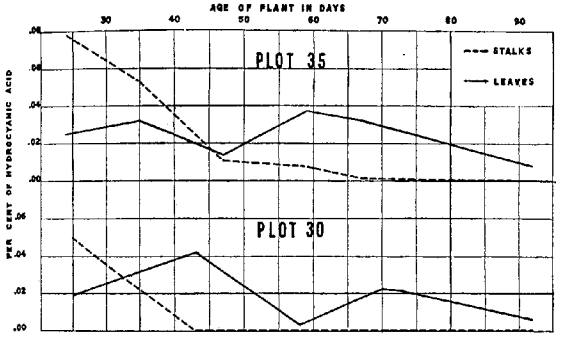

FIG. 2.—Curve showing the distribution of hydrocyanic acid in sorghum. The percentage of hydrocyanic acid is based on dry matter.

nitrogen is deficient the plant maintains the equilibrium of other nitrogenous compounds at the expense of the prussic acid.

Figure 2 shows the distribution of the prussic acid in the two varieties of sorghum, and an interesting varietal difference appears. The stalks of feterita contain in the early stages a relatively high percentage of prussic acid, and the acid persists in small amount through most of the life of the plant. The stalks of Orange sorgo, however, show less of the acid in the beginning, and it disappears entirely by the fortieth day. Both plots show an increase of the acid in the leaves during the early stages, and later a decrease; the acid in the leaves had not completely disappeared in this experiment by the ninety-second day. Evidently the cyanogenetic glucosid is related to the vital processes of the plant, as it occurs in the largest quantity in those parts of the plant which are most active photosynthetically and during those stages when the plant

is developing most rapidly. Treub (12) and Ravenna and Zamorani (9) are of the opinion that hydrocyanic acid is a necessary intermediate product in the formation of proteins. As such, the quantity present at any one time might be subject to such variation as this experiment shows.

Further experiments will be carried on next season (1915) to determine, if possible, just what effect variety and climatic conditions may have on the prussic-acid content of sorghum, as well as the function of the acid in the metabolism of the plant.

METHOD OF DETERMINING HYDROCYANIC ACID

For the determination of hydrocyanic acid the colorimetric method of Francis and Connell (7) was used, with one important modification. It was found that when the macerated tissue was distilled with sulphuric acid according to their method the distillate became yellow, and when subsequently treated with ferric chlorid a greenish or brownish precipitate was formed which masked the color of the thiocyanate. Enzym hydrolysis was therefore resorted to. Slade (11) digested the ground tissue for 12 hours at room temperature, making use of the enzym which is always found in a plant in conjunction with a cyanogenetic glucosid. But we found that at 40° to 45° C. complete hydrolysis was obtained in two hours or less, as portions of the same sample gave the following results:

Time of digestion.	HCN in 10 gm. of ground material.
2 hours	0.00040 gm.
4 hours	0.00040 gm.
6 hours	0.00025 gm.

In all our work 2-hour digestions were used, and the hydrocyanic acid distilled and determined in the usual way.

CONCLUSIONS

The following points may be presented as a summary of these notes:

(1) When sorghum is grown on poor, infertile soil, added nitrogen may slightly increase the amount of hydrocyanic acid in the plant. With a fertile soil and abundant nitrogen this effect may not be produced.

(2) During the first three or four weeks of the plant's life the prussic acid is concentrated in the stalks. Then it rapidly decreases and disappears there, but apparently persists in the leaves in decreasing percentages until maturity.

(3) Climate and variety may be more important factors than soil nitrogen in determining the amount of the acid in the plant.

(4) Complete hydrolysis of the glucosid is obtained by digesting the macerated tissue for two hours at 40° to 45° C.

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EFFECT ON SOIL MOISTURE OF CHANGES IN THE SURFACE TENSION OF THE SOIL SOLUTION BROUGHT ABOUT BY THE ADDITION OF SOLUBLE SALTS

[A PRELIMINARY REPORT]

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While temporary research assistant in soils in the Michigan Experiment Station the writer had occasion to do laboratory work in which information was sought as to how far the effect of fertilizer or soil-amendment materials in altering the moisture condition of soil is dependent upon changes produced in the surface tension of the soil solution.

The effect of the materials on the surface tension when in dilute water solutions was taken as an indication of their effect on the surface tension of the soil solution. The surface tension of the dilute solutions, together with their viscosity, specific gravity, and resistance in ohms, is shown in Table I. For comparison like expressions for pure water and for two soil percolates are also introduced.

TABLE I.—*Effect of salts on certain physical properties of the solutions*

Solution.	Specific gravity.	Resistance.	Surface tension, 25° C.	Specific viscosity.
		Ohms.	*Dynes.*	
Water	1.0000	[a] 180,000.0	72.00	1.0000
$NaNO_3$	1.0066	87.8	72.33	1.0049
$(NH_4)_2SO_4$	1.0059	65.5	72.15	1.0163
Manure extract	1.0059	89.5	59.13	1.0992
NaCl	1.0070	57.6	72.36	1.0200
KCl	1.0063	59.2	72.24	1.0111
K_2SO_4	1.0080	82.0	72.29	1.0132
$CaH_4(PO_4)_2$	1.0060	222.3	72.36	1.0207
Na_2CO_3	1.0099	71.8	72.15	1.0464
Sandy-loam percolate	1.00257	224.0	70.75	1.0595
Clay-loam percolate	1.00293	190.0	71.18	1.0677

[a] Approximately.

The solutions of the single salts were made by dissolving 10 gm. of Kalbaum's chemically pure salts in 1,000 c. c. of pure water. The changes brought about here in the physical properties of the solutions very probably were greater than those which result in the soil solution from average field application of the salts. The manure extract was obtained by forcing out the liquid contents of approximately equal parts of fresh solid and liquid horse manure and then diluting to about

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the resistance of the sodium-nitrate solution. The soil percolates were obtained by taking the first small portions of solutions percolated through 500 gm. of previously air-dried soil in a percolator tube. The first 15 c. c. were used from sandy-loam soil and the first 20 c. c. from the clay-loam soil. These portions were the densest percolates it was possible to obtain.

All the single salts increased the surface tension to some extent, but in no case was this action marked.[1] The viscosity was also increased in all cases. Changes in the viscosity of the soil solution, while theoretically not affecting the final distribution of moisture reached, should, nevertheless, alter the time required for this state to be gained. The small increases in surface tension noted above could hardly be expected to exert any appreciable effect on the moisture content of, or the moisture movement in, soil. In this connection the work of Whitney[2] in determining the effect of soluble salts on the surface tension of solutions, which has been used to show that fertilizer salts may exert a significant effect on the soil-moisture content or movement through changes in the surface tension, seemingly has been allowed more weight than it merits. The solutions used by Whitney were either very dense or else saturated, and as such gave changes in the surface tension that would not be comparable with those arising from the field application of fertilizer salts. The surface tension of the manure extract was much lower than that of water. It is interesting to note that the surface tension of the soil percolates was but little lower than that of water.

Information as to the effect of the materials on the soil-moisture content and movement was secured through the following experimental work. Samples of the sandy-loam and the clay-loam soils from which samples for the soil percolates were obtained were weighed out. The sandy-loam samples weighed 660 gm. each, and the clay-loam samples, 580 gm. each. Fifty c. c. of the dilute water solutions above described, omitting the two soil percolates, were added to each sample. This is at the rate of 1,000 pounds per acre of surface, with the exception of the manure extract. The treated samples were air-dried, mixed, and put into glass tubes 6 cm. in diameter and 20.32 cm. in height. In each case the samples were packed into a volume of 505 c. c., making a soil column approxi-

[1] Values for the surface tension and also for the viscosity of these salts in some cases of densities not far removed from that employed here have been determined by a number of investigators in pure physico-chemical lines. (See Castell Evans, John. Physico-Chemical Tables for the Use of Analysts, Physicists, Chemical Manufacturers, and Scientific Chemists . . . v. 2, p. 756. London, 1911.) The results given here are not presented as affording any essentially new information along this line. The work was done largely for the purpose of establishing the accuracy of the work to follow on soil percolates and manure extract solutions.

The drop method was used in the surface-tension work, employing the dropping pipette (stalagmometer) of Traube. (See Abderhalden, Emil. Handbuch der biochemischen Arbeitsmethoden. Bd. 5, T. 2, p. 1358. Berlin, Wien, 1912.)

[2] Whitney, Milton. Some physical properties of soils in their relation to moisture and crop distribution. U. S. Dept. Agr. Weather Bur. Bul. 4, 90 p. 1892.

mately 18 cm. in height. The columns were saturated with water, sealed at the tops, weighed, and placed on an air-dried sandy-loam soil. From time to time they were removed, weighed, the moisture content thus determined, and replaced. There was no covering over the bottoms of the tubes, so that the soil columns were in direct capillary contact with the dry soil underneath. The amount of moisture in this undersoil varied from 1 to 3 per cent throughout the experiment. The treatments were run in quadruplicate with each soil. In addition to the treatments already mentioned, there were four check or no-treatment columns with

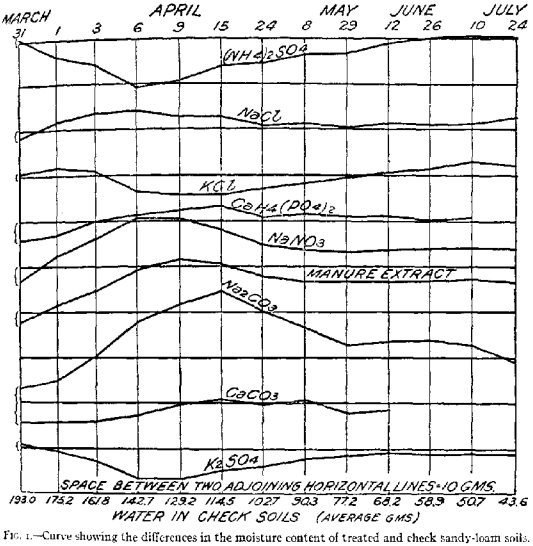

FIG. 1.—Curve showing the differences in the moisture content of treated and check sandy-loam soils.

each soil, and likewise four columns to which 2 gm. of calcium carbonate, at the rate of 4,000 pounds per acre surface, had been added.

The differences in the moisture content throughout the experiment between the treated soils and the check soils are shown in the accompanying curves (fig. 1 and 2). Here the amount of water in the check soils is represented by a horizontal line and the increase or decrease of water in the treated soils over or under this by distance above or below these lines.

The effect of the treatments on the moisture content or water-retaining power of the soils is summarized in Table II.

TABLE II.—*Effect of various fertilizer salts on the moisture content of soils*

Treatment.	Water-retaining power.		
	Increased with—	Not affected with—	Decreased with—
Na_2CO_3	Sandy loam Clay loam		
Manure extract	Sandy loam Clay loam		
$NaNO_3$	Sandy loam Clay loam		
NaCl	Sandy loam Clay loam		
$CaCO_3$		Sandy loam Clay loam	
$CaH_4(PO_4)_2$		Sandy loam Clay loam	
KCl		Clay loam	Sandy loam.
K_2SO_4		Clay loam	Sandy loam.
$(NH_4)_2SO_4$		Clay loam	Sandy loam.

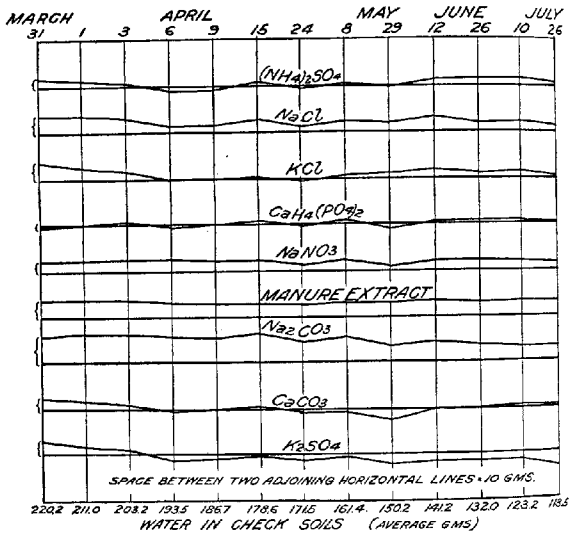

FIG. 2.—Curve showing the differences in the moisture content of treated and check clay-loam soils.

It has already been said that the effect of the single salts on the surface tension of the solutions was too small for any measurable changes in the moisture condition of soil to be expected from this source. In the case

of the manure-extract solution, however, there was a marked decrease in the surface tension, but this change evidently exerted no apparent effect upon the moisture content of the soils treated with the manure-extract solution, for these soils showed an increase in the moisture movement rather than a decrease, which would have resulted from any noticeable action of a decreased surface tension.

The fact that the treatments which influenced to any extent soil-moisture content or movement are those known to have a marked influence on the physical structure of soils points very strongly to the conclusion that herein lies the effect of the salts in this regard. Some information as to the effect of the treatments on the physical structure of the soils in this experiment was obtained by the following work. At the close of the moisture work the soil columns were removed from the tubes and allowed to air-dry. Those found to be intact were selected and equal lengths of the columns, 2½ inches, were removed from their lower ends. The resistance of these sections to a crushing force was determined. The results are shown in Table III.

TABLE III.—*Resistance of sections of the soil columns to a crushing force*

SANDY LOAM

Treatment.	Resistance.	Average resistance.	Treatment.	Resistance.	Average resistance.
	Kilos.	*Kilos.*		*Kilos.*	*Kilos.*
Na_2CO_3	19. 91	19. 91	K_2SO_4	12. 68	12. 83
$NaNO_3$	16. 48	16. 48		12. 98	
Manure extract	16. 36	16. 24	KCl	13. 06	12. 54
	16. 12			12. 01	
NaCl	14. 25	15. 28	$CaH_4(PO_4)_2$	11. 97	11. 96
	16. 31			12. 51	
$CaCO_3$	14. 87	14. 24		11. 39	
	15. 37				
	12. 47				

CLAY LOAM

Na_2CO_3	92. 78	91. 43	$CaHPO_4$	73. 27	74. 90
	90. 08			75. 81	
$NaNO_3$	88. 81	88. 81		73. 27	
	86. 83			77. 24	
NaCl	82. 80	85. 88	$CaCO_3$	68. 68	68. 68
	87. 07		KCl	67. 88	67. 88
	86. 83			67. 00	
Check	75. 73	80. 37	K_2SO_4	67. 09	67. 56
	85. 01			68. 60	
$(NH_4)_2SO_4$	79. 78	75. 87			
	74. 08				
	73. 75				

A comparison of the order of treatments here with that in Table II, in which the effect of the treatments on moisture content or movement is given, shows close correlation between the two. Treatments which

detrimentally affected the structure of the soil and made the soil more close evidently retarded the moisture movement and increased the moisture content, while treatments which promoted the soil structure and made the soil more open hastened the moisture movement and decreased the moisture content.

The results from the work presented in this paper indicate that changes in the surface tension of the soil solution arising from application of fertilizer salts are of no importance in affecting the moisture condition of the soil.

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